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CONTRIBUTIONS FROM THE CRYPTOGAMIC LABORATORIES OF HARVARD UNIVERSITY.

No. LXX.—*CULTURE STUDIES OF FUNGI PRODUCING BULBILS AND SIMILAR PROPAGATIVE BODIES.*

By John William Hotson.

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By John William Hotson.

Presented by Roland Thaxter. Received June 19, 1912.

Contents.

Introduction.

The term "bulbil" was first employed in connection with Fungi by Eidam in 1883 to designate certain sclerotium-like bodies, somewhat definite in form, and capable of reproducing the plant. They vary greatly in appearance, some consisting of a compact mass of homogeneous cells clearly distinguished from certain others which surround them. The latter form a single layer or in some cases several layers of cells, which may or may not become empty and colorless and which correspond, in a general way, to the pseudospores or accessory spores of certain smuts, while the cells which they surround are functional spores and capable of germination. Bulbils are the predominant type of reproduction in certain fungi, and in some cases the only means at present known. The most typical bodies of this nature are readily distinguished from sclerotia by their smaller size, more definite structure, and peculiar methods of development. There are other types, however, that seem to approach more nearly true sclerotia; while others again resemble very closely the "spore balls" of such forms as Tuburcinia, Urocystis, etc., among the Ustilaginales, or even the compound spores of such forms as Stemphylium, Mystrosporium, etc., among the Hyphomycetes; but from the first they are definitely distinguished by their method of germination, while in general they are readily separated from the last two by their mode of development. They thus seem to possess morphological characters that would place them in an intermediate position between sclerotia, on the one hand, and compound spores of the dictyosporic type on the other, with examples of transitional forms which grade into the former and others that are almost indistinguishable from the latter.

Bulbiferous conditions among the fungi have, in general, been described under the following genera of the so-called "Fungi Imperfecti": Papulospora, Helicosporangium, Baryeidamia and Eidamia;

but in a few instances, in which their association with other and more definite types has been reported, they have been included under the generic name applied to the latter as, for example, Dendryphium or Haplotrichum. There seems to be little or no uniformity or agreement among the writers on this subject, especially among the earlier ones, regarding the morphological significance of bulbils. Preuss, who was the first to describe bodies of this nature in 1851, considered each bulbil a single compound spore and placed the genus Papulospora, which he had created for their reception, in the "Bactridiaceae" of Corda, a family not now recognized, which was established to include fungi like Trichocladium Harz, bearing compound spores and with prostrate fertile hyphae. On the other hand, Karsten ('65) regarded the bulbil-like bodies which were associated with his "Helicosporangium" as an ascus-producing structure, which was included by him among the Erysipheae. Again, Eidam ('83) was of the opinion that the two genera, Papulospora and Helicosporangium, occupied an intermediate position between Ustilagineae and Erysipheae, while E. Fischer is inclined to place them among the Monascaceae. De Bary, in his "Morphology and Biology of Fungi," considers them briefly and includes them in a category which he calls "Doubtful Ascomycetes" and suggests that "the plants should be further investigated." In considering these forms at a later period, Harz ('90) included all structures of this nature then known under a new order, the "Leptoomycetes" and expressed the opinion that they are somewhat closely related to the Oomycetes and coordinate with them and the Zygomycetes.

Inasmuch as these bulbils have received very little attention, our knowledge of their morphology, development, and taxonomy is very meagre. These forms are not as rare as has been generally supposed but are, on the contrary, widely distributed and of common occurrence. Substrata which have produced bulbils have been obtained from various parts of Canada and the United States; from Guatemala, Mexico, and West Indies; from South America and Europe. Their small size, the nature of the substratum on which they grow, and their failure to form a conspicuous fructification in a majority of cases, account to some extent for the fact that they are generally overlooked in the field and in laboratory cultures.

The results of the present investigation emphasize the fact, more recently brought out by several mycologists, that these fungi do not belong to any one of the Natural Orders, nor do they in any sense form a group by themselves, but occur without regularity as imperfect

forms among the main groups of Higher Fungi. The forms associated with bulbiferous conditions which are herewith enumerated include among the Discomycetes, a new species of Cubonia; among the Hypocreales, three species of Melanospora; and among the Basidiomycetes at least four types; while nine species of Papulospora as yet unconnected with a perfect form are added to those already known. Among the latter also, *Papulospora candida* Sacc. has been found to be associated with a second and well marked imperfect form, namely *Verticillium agaricinum* var. *clavisedum*. In the life histories that have been worked out, the results have been obtained from pure cultures which, in many cases, have run for a number of years, and care has been taken to avoid any errors resulting from contamination.

In view of the very general occurrence of bulbils, it is somewhat surprising that more attention has not been given to them. The literature on the subject is quite limited and the accounts given often conflicting. Preuss, Karsten and Eidam did their work at a time when Mycology was in a more or less transitional condition, the modern bacteriological methods had not yet been applied to the cultivation of fungi, a fact which may account to a certain extent for the varied and often conflicting opinions of these earlier writers. Certain more recent contributions, however, have given us more accurate information as to certain isolated forms and the investigations of Mattirolo, Berlese, Bainier and Lyman have suggested or demonstrated the actual relationships of certain forms to species among the Ascomycetes and Basidiomycetes, of which they prove to be imperfect conditions. There has been no attempt, however, so far as the present writer is aware, to investigate the general subject of bulbiferous fungi.

The need of further examination of the morphology and development of bulbils was suggested by Professor Roland Thaxter, under whose direction and supervision the work has been conducted. The problem was begun and finished in the Cryptogamic Laboratories of Harvard University, some culture work and collections of material being done in California while the writer was connected with Pomona College.

It is a pleasant duty for the writer to acknowledge, at this point, his indebtedness to those who have rendered him assistance in carrying on this research: especially to Professor Thaxter are grateful acknowledgments due, for suggestions, kindly advice and encouragement, and for placing at the writer's disposal many dried specimens and tube cultures of bulbils which had been collected by him, and for

the use of a number of papers belonging to his private library; to Professor Elias J. Durand of the State University of Missouri, for the description and naming of *Cubonia bulbifera;* to Professor W. G. Farlow for material and the use of several articles from his private library.

Review of Literature.

The literature relating to bulbils is, as has been already indicated, by no means extensive, and deals with less than a dozen described forms, some of which do not appear to have been recognized by mycologists since their original publication. In order to give a clearer idea of the present state of our knowledge of the subject, it seems desirable, before proceeding further, to give a brief summary of the more important papers, which may be conveniently considered seriatum under the following heads:

(a) Helicosporangium, (b) Papulospora, (c) Pyrenomycetous Forms, (d) Discomycetous Forms, and (e) Basidiomycetous Forms.

(a) *Helicosporangium.*

The genus Helicosporangium was first described by Karsten ('65) and was based on a form said to be "parasitic" on beet roots, which he named *H. parasiticum.* According to his description the fertile branches of this fungus tend to become erect, and are septate like the rest of the hyphae. In the process of development they coil up spirally at the end to form the bulbil. This character suggested that they might be closely related to such hyphomycetous forms as Helicoma Corda, Helicosporium Nees, Helicomyces Lk., Helicotrichum Nees, etc. In fact, it was this spiral development of the fructification, held in common with these forms, that suggested to Karsten the name, Helicosporangium.

At maturity these bulbils are described as almost spherical, with one large central cell which is surrounded by a single layer of colorless cortical cells which form a complete wall. Karsten believed that one of these cortical cells produced a short protuberance on the inner side, which extended into the large central cell, in which he says a "nucleus" soon appeared and enlarged quite rapidly. He further observed that the contents of the central cell soon became somewhat differentiated and divided into a number of small cells, usually eight in number, but varying from seven to ten, which gradually enlarged to form free, hyaline, elliptical spores; and, after escaping from the

central cell, divided, forming compound spores of two cells. On germination each cell produced a germ tube.

Karsten believed that the contents of the cortical cells entered directly, or by diffusion, into the large central cell and that only after the contents intermingled were the spores formed. This suggested the possibility of sexual differentiation of certain cells which made up the coil, the end-cell, in his opinion, acting as an oogonium and the second or even the third or fourth cell acting as an antheridium.

It will thus be seen that in Karsten's opinion the peculiar structures which he described in Helicosporangium were neither bulbils nor homologous with other non-sexual propagative bodies, and although it is possible that he may have been dealing with some form allied to Monascus, in which a sexual process was actually present, it seems not improbable that he was misled by what he saw. Since, however, this subject will be further discussed below in connection with a form which appears to be identical with Karsten's species, it need not be further considered in the present connection.

Eidam ('77, '83) described and figured a bulbil obtained from moist turnips which he referred to *Helicosporangium parasiticum* Karsten, but, as has been pointed out by Karsten himself ('88), Harz ('90), and others, it seems probable that he was dealing with a fungus different from that which Karsten described. Eidam's fungus is said to be saprophytic, producing numerous conidia borne on characteristic bottle-shaped sterigmata and having two kinds of bulbils which do not contain endospores. In these respects it is said to differ from that described by Karsten. This matter, however, will be referred to again below.

De Bary ('87) accepted in general the views expressed by Eidam ('83) regarding *H. parasiticum*, but Karsten ('88) maintained that he did so because he had not read the original article, but formed his opinion on information obtained from "Eidam's unfortunate review of it" ('83), and in conclusion ironically gives the name Baryeidamia to Eidam's fungus, in recognition of what he considered the combined blunders of these two mycologists in dealing with this form.

A third species referred to the genus Helicosporangium was described under the name of *H. coprophilum* by Zukal ('86) and was found by him on horse dung associated with *Stysanus stemonites* Cd. According to Zukal's description, this bulbil consists of two to eight large central cells with thick walls of a dark-red color, which are surrounded by a layer of smaller cortical cells of a lighter color. The form and manner of development of this bulbil are said to vary con-

siderably. "Indeed," he says, "there are hardly two to be found which are exactly alike."

Zukal ('86) also describes a yellowish-brown bulbil under the name of *Dendryphium bulbiferum*, found on birch twigs, the mycelium of which is said to grow up, tree-like, and to branch monopodially, the ultimate branches terminating in rows of small hyaline ellipsoidal cells. At maturity these little cells become brownish and, when they are abstricted, form a dusty mass. The bulbil associated with them is almost spherical and bears a very close resemblance to *Helicosporangium parasiticum* Karsten, both in its mode of development and in its general appearance.

On decayed fruit of *Lycopersicum esculentum* Mill. Zukal ('86) has reported the occurrence of bulbils closely resembling, both in appearance and development, the two types above referred to, but which are said to differ in their greater variations and irregularities, and also in the fact that they are associated with the conidia of *Haplotrichum roseum* Lk. (*Oedocephalum glomerulosum* Bull.). It should be mentioned in this connection, however, that since Zukal did not apparently deal with pure cultures and no such bulbils have been found, as far as the writer is aware, by others who have cultivated this very common Hyphomycete, his statements must be accepted with some reserve. It may be stated at this point that in none of the published accounts of Helicosporangium is there any evidence that pure cultures were used, and thus the possibility of contamination renders these results largely untrustworthy.

(b) *Papulospora*.

Of the several species which have been placed in this genus the first was described by Preuss ('51) from material found growing on decayed pieces of apple and was said to be connected with chlamydospores which resembled those of Sepedonium. He therefore named his species *P. sepedonioides*. These bulbils are described as irregularly arranged on lateral branches, white at first and later becoming rust-colored, with the cortical cells differentiated from the central ones. Preuss regarded this bulbil as a single multicellular spore and not as a cluster of single spores, because they never break up into individual cells, although he thought the cortical layer probably bursts at the time of germination.

Eidam ('83), in the paper already referred to, described a second bulbil found quite abundantly on straw, weeds, dung, etc., which

appeared, in his opinion, to be so closely related to the form described by Preuss that he placed it in the same genus; since it was, however, not associated with chlamydospores like those of Sepedonium, but with an Aspergillus-like fructification, he named it *P. aspergilliformis*. Two kinds of bulbils were described as connected with this fungus, which resembled each other in color but differed in their mode of development. Of these two types, one is said to be large, sclerotium-like, without any differentiation into central and cortical cells, while the other is small and consists of several large central cells surrounded by a row of colorless cortical cells resembling those of *Helicosporangium parasiticum*, mentioned in the same paper.

In connection with this fungus Eidam described conidia which, he states, were produced on exceedingly delicate, colorless, conidiophores resembling somewhat those of *Aspergillus albus* Wilhelm, but the sterigmata are usually flask-shaped. These conidia were also borne individually on the sides of ordinary hyphae, being abstricted in chains from flask-shaped sterigmata and resembling those described by Eidam as associated with the form which he referred to *Helicosporangium parasiticum*.

"Chlamydospores" were also described by Eidam in connection with his *P. aspergilliformis*. "This form of reproduction," he says, "seems to be by far the most common one connected with Papulospora and often is the only one. I have found, in great abundance, mycelia with only chlamdospores and no trace of bulbils or conidiophores." On account of the presence of these chlamydospores which resemble the spores of Acremoniella, Lindau ('07) has redescribed this species under the name of *Eidamia acremonioides* Harz. The criticism that was offered as to the reliability of Eidam's investigation of Helicosporangium may equally well be applied here. Bainier ('07) is of the opinion that he mistook the conidia of *Acremoniella atra* Sacc. (*Acremonium atrum* Corda) for chlamydospores belonging to Papulospora, as these two species are often found associated with each other.

Bainier ('07) found a fungus abundantly on straw, paper, cardboard, etc., which he calls *P. aspergilliformis*. His description of the conidia and conidiophores is practically the same as that given by Eidam ('83). His fungus, however, does not produce acremonium-like chlamydospores, as did that of Eidam, but, on the other hand, developed paritheeia with long necks, which he refers to the genus Ceratostoma. The asci, which are very transitory, even disappearing before the maturity of the spores, are ovoid with eight simple brownish spores

somewhat variable in shape and grouped together, forming a sort of ball. Moreover, he considers that the bulbils of *Helicosporangium parasiticum* described by Eidam are merely abnormal forms of *P. aspergilliformis*, such as are often found among other Mucedineae.

Another Papulospora, which was found in the tubers of Dahlia, has been described under the name of *P. dahliae* by Costantin ('88). The bulbils of this fungus are spherical, brownish-red in color, with two or three large central cells. All the cells are said to contain granular protoplasmic material at first, but the central cells soon become strongly colored violet and more densely filled with granular material and oil globules, and eventually the peripheral cells become empty and transparent. There were found associated with this fungus colorless septate spores which taper at both ends and correspond very closely to those described by Saccardo (Michelia II, p. 20) under the genus Dactylaria. Here again there is little evidence that the investigation was carried on with pure cultures and it is doubtful that the conidia and the bulbils described belong to the same fungus, since they were only found associated and not actually connected. It would thus appear that the only contribution on Papulospora that shows any evidence of work with pure cultures is that of Bainier ('07).

(e) *Pyrenomycetous Forms.*

The first evidence of the definite association of a bulbil with one of the Pyrenomycetes as an imperfect form, is found in the description of *Melanospora Gibelliana*, published by Mattirolo in 1886,— although Zukal ('86) a few months previously had announced that he had found bulbils in connection with *Melanospora fimicola* Hansen, and *M. Zobelii* Corda, but gave no description of them. The fungus studied by Mattirolo was found growing abundantly on decayed chestnuts and was said to produce not only perithecia of Melanospora but also bulbils, conidia and chlamydospores. In appearance and development these bulbils are said to resemble closely those of Baryeidamia, but with more variations. Their color is pale yellow when young, brownish-yellow at maturity, and they are often 100 μ in diameter. Mattirolo considered them immature perithecia, but, although he employed the most varied methods of experimentation, he was unable to make them develop into melano-porous perithecia. The conidia said to be connected with this fungus are described as small, colorless, spherical spores, on bottle-shaped sterigmata, resembling closely those mentioned by Eidam as belonging to Baryeidamia.

The chlamydospores referred to this fungus are said to have very rough, thick walls, resembling somewhat those of Sepedonium. Although Mattirolo is of the opinion that these chlamydospores form a phase of the life history of *M. Gibelliana*, he admits that he has not absolutely proven it. He states he has "cultivated these forms without ever being able to establish unquestionably their origin and relation."

Berlese ('92) described a bulbiferous fungus producing perithecia, which he named *Sphaeroderma bulbilliferum*. This fungus he found growing abundantly on dead leaves of Vitis, Cissus and Ampelopsis. It is said to have several modes of reproduction, such as (a) microconidia, which appear in chains and which resemble those figured by Mattirolo as belonging to *Melanospora Gibelliana* and by Eidam, to *Helicosporangium parasiticum*; (b) chlamydospores, which varied somewhat in size — (these were ovoid, usually smooth, and golden-yellow in color, each with a septum near the base, which divided the chlamydospore into two unequal cells); (c) golden-yellow bulbils, which resembled those described and figured by Mattirolo in *Melanospora Gibelliana* and which seem to be short-lived and, under the most favorable conditions, could not be made to produce mycelia; (d) perithecia, which were represented as almost spherical and when mature measured from 400–500 μ in diameter. They remain without an ostiole almost to maturity and consequently there is no formation of a neck. The color of the young perithecium is yellowish but becomes darker as it grows older, until at maturity it is almost a tan color. The asci are club-shaped with deep smoke-colored spores, ovoid and prolonged at the poles into short obtuse papillae.

Another pyrenomycetous form producing bulbils has been reported by Biffen ('01, '02), and is said to be connected with *Acrospeira mirabilis* Berk., which was originally found on sweet chestnuts (*Castanea vesca*, Gaertn.). By the use of pure cultures, Biffen claims to have succeeded in obtaining not only the chlamydospores, as described by Berkeley and Broome in the Annals and Magazine of Natural History for 1861, but also what he calls "spore-balls" (bulbils) and definite perithecia.

The spore-balls, which he says so closely resemble *Urocystis violae* that he "could not find a single characteristic to separate them by," were obtained by sowing the 'chlamydospores' on a watery extract of chestnuts. Greater difficulty was experienced in producing the perithecia, but finally, by sowing the chlamydospores and bulbils on sterilized chestnuts, he records the following results: — "The 'chlamy-

dospore' infections gave a crop of 'chlamydospores' only; the spore-balls gave spore-balls and small reddish-brown, hard-walled perithecia. The walls of the perithecia were smooth and without bristles and the ostiole was small and flush with the surface, i. e., not raised on a papilla or forming a neck . . Berkeley's *A. mirabilis* thus turns out to be one of the stages in the life history of a Sphaeria."

The investigations on the pyrenomycetous forms show more careful work than those under the two preceding headings. In all these there is evidence that pure cultures were used more or less, but in most cases it is uncertain how far the results were thus obtained.

(d) *Discomycetous Forms.*

There have been two fungi described which produce bulbils associated with discomycetous fructifications, one by Zukal ('85, '86) and the other by Morini ('88). Zukal found two kinds of primordia in connection with his fungus; one, he says, consisted of two or three small mycelial branches which wound about each other and eventually produced reddish-brown bulbils with a cortex of small colorless, almost transparent, cells. The other primordium was made up of a number of hyphae massing themselves together and becoming quite large and, under proper conditions of nutrition, developing into apothecia of the Peziza type; but he does not give a name to this form. This fungus produced conidia abundantly on erect, branched conidiophores. The conidia are spoken of as colorless, ellipsoidal, smooth, and they appear in clusters upon the ends of short sterigmata. Zukal's cultures were grown on absorbant paper saturated with Leibig's extract, but there is no evidence in his article that these were pure cultures, or that the life history of the fungus was carefully traced from ascospore to bulbil.

Morini ('88) describes "bulbil-like" bodies associated with *Lachnea thelebloides* (A. & S.) Sacc. in old cultures. Since these occurred only in cultures that had run for a long time, in which the nutrient was probably largely exhausted by the previous growth of the fungus, and since the development was largely the same as that of the apothecium, Morini considers that the bulbils of *L. thelebloides* are abortive apothecia and, further, that they are analagous to the similar structures described by Eidam, Karsten, et al. He apparently has used pure cultures in his investigation, but to what extent his results were obtained from such cultures could not be determined from his paper.

(e) *Basidiomycetous Forms.*

The only account, as far as the writer is aware, of the definite association of bulbils with Basidiomycetes is given by Lyman ('07) in connection with his culture-studies of *Corticium alutaceum* (Schrader) Bresadola, his results having been obtained from pure cultures made of the basidiospores of this fungus. "The bulbils," he says, "are reddish-brown or chocolate-colored clusters of cells, more or less globose in shape, and usually 65–80 μ in diameter, although ranging as high as 220 μ. . . . They are frequently very irregular in shape, due to the unsymmetrical arrangement of the cells, and to the bulging of the free outer walls. There is no distinction between internal and external cells of the cluster." Besides the basidiospores and bulbils this Corticium also produces conidia which are of the Oidium-type. Occasionally whole hyphae break up into chains of spores of this type.

Lyman also mentions two other bulbiferous fungi which were referred to the Basidiomycetes, being recognized as such by the clamp-connections of their hyphae, although the basidiospores were not obtained.

Lastly, it may be well to mention an article by Harz ('90), in which he describes a fungus found growing on material obtained from the reservoir of a factory and which he names *Physomyces heterosporus* (*Monascus heterosporus* (Harz) Schröter). Although this fungus is probably a true Monascus, as Schröter has indicated, yet since it has been associated with bulbils, and since the ascocarps of Monascus in general bear a superficial resemblance to them, it may be well at least to mention it in passing. Harz has associated this form closely with *Helicosporangium parasiticum* Karsten, and created a new family — Physomycetes — for the reception of these two genera. As, however, these two forms will be referred to again in connection with *H. parasiticum* Karsten, a further consideration of them will be deferred until that time.

It will be seen from the foregoing brief review of the literature that much of it is quite vague and untrustworthy. This perhaps is what one would expect from investigations which were carried on during a period prior to the adoption by mycologists of the bacterial methods of handling pure cultures. This is especially true with regard to polymorphic forms, like some of those under consideration, where it is so necessary to adopt these methods in order to be absolutely sure of the different steps in following the life history of the fungus from spore-form to spore-form. The contributions of Lyman and Biffen

on this subject show undoubted evidence that their investigations were carried on with pure cultures and that the life history from spore to bulbil was closely traced. It is probable that Bainier, Morini, Berlese, and Mattirolo also used pure cultures more or less, but there is little evidence in their writings that there was careful tracing of the fungus from spore to bulbil.

Sources of Material.

Before recording the results obtained from the study of the various bulbiferous fungi cultivated by the writer, it will be well to refer briefly to the sources of material and the methods used in this investigation.

In 1907, at the suggestion of Dr. Thaxter and with a view to obtaining as much material as possible for examination, the writer began collecting substrata of various kinds from widely different localities. This material was placed in moist chambers in the laboratory and as bulbils appeared pure cultures were made of them. The methods employed in doing this will be referred to later. Most of the material from which bulbils were obtained was collected either in the vicinity of Cambridge, Mass., or Claremont, Calif.; but bulbils were also procured from substrata received from other portions of New England and California, from Kentucky, Canada, Mexico, Guatemala, Cuba, Jamaica, Bermuda Islands, the Argentine Republic, Italy, etc.

The substrata on which these fungi were found were very diverse. The most productive were various kinds of excrement (dog, rat, mouse, rabbit, pig, horse, goose, goat, etc.), dead wood (Acer, Lathyrus, Quercus, Eucalyptus, etc.), decaying vegetables (squash, onions, etc.), straw (wheat, oats, barley, rye, alfalfa, etc.). A number were found on paper and old cardboard, as well as on a variety of other substrata. Of many hundreds of such cultures about two hundred yielded bulbils.

Culture Methods.

The moist chambers used for the cultivation of these materials were usually crystallizing dishes covered with pieces of glass. A large amount of this material was grown in the laboratory and from time to time was carefully examined through the glass top with a hand lens. When bulbils were observed, one of them was picked out by means of fine dissecting-needles under a dissecting microscope, and after thorough washing in sterilized water on a flamed slide, was transferred to a test-tube containing sterilized nutrient material — usually potato

agar. In the case of some melanosporous forms the transfer was made by carefully touching the long cirri of ascospores, produced by the perithecia of this genus, with a piece of nutrient agar on the end of a sterilized platinum needle. The ascospores adhering readily to the agar, a pure culture was easily obtained.

Bacteria sometimes gave trouble in some transfers, but as a rule these were gotten rid of either by picking out separate bulbils carefully and washing several times before growing them in acidulated nutrient agar, or by keeping the impure tubes at a temperature of 15–20° C. The growth of the bacteria being retarded either by the cold or acid, the mycelium producing the bulbil soon grew out beyond the affected region, and by gouging out a few of the ends of the hyphae with some of the agar and transferring to another tube, a pure culture was readily obtained.

When these were secured the fungus was cultivated on various kinds of nutrient agar media, some growing better on one medium and some on another. The following were used most frequently: potato, onions, sucrose of different percentages, bran, rice, cornmeal, straw, plums, prunes, grapes, figs, bread, squash, Spanish chestnuts, wood, various kinds of dung, etc. These were usually used with agar, but some materials like wood, dung, straw, nuts, etc., were sterilized in bulk with plenty of water and without using agar while in some instances decoctions were used. In Claremont, California, they were grown in the laboratory at an average temperature of 25–30° C. In Cambridge many were grown in an oven kept at various constant temperatures, 20–25° C. giving the best results.

The vessels used for these cultures were usually medium sized test-tubes, Erlenmeyer flasks of one and two litres, or preserve-jars with cotton plugs. These were filled about one-third full of nutrient agar and usually slanted to give more surface. On this nutrient the fungus would usually grow well for several months, and results were often obtained from pure gross cultures which could not be secured from the smaller ones.

In the germination of the spores and bulbils, Van Tieghem cells were used very freely. For this purpose cover glasses of one inch and two inches in diameter were used and carefully sealed, plenty of sterilized water having previously been put in the cells which corresponded in dimensions with that of the cover glasses. The large Van Tieghem cells afforded an opportunity of using cultures of considerable size which were usually composed of decoctions of different kinds of nutrient material, sometimes with agar to make them solid, while at other times the decoctions were used as hanging drops.

In cases where the transfer of conidia, only, was desired, two methods were employed to avoid getting either bulbils or pieces of mycelium. If the conidia were quite plentiful or were on erect stalks so that they were somewhat separated from the rest of the mycelium, this could be accomplished by means of a piece of nutrient agar on the end of a sterilized platinum needle. By careful manipulation and with the aid of a dissecting microscope, they could be touched with the agar to which they adhered readily, and after examination under a microscope to determine if there were only conidia present, they were immediately transferred to a new tube or a Van Tieghem cell, as the case required. In instances where the above method could not be used, or where cultures from individual conidia were required to verify the relation between a conidial form and the bulbil, Barber's spore-picking apparatus ('07) was employed. Plate-cultures were also used to advantage in some instances for separating the conidia from the bulbils.

Throughout this investigation, as already stated, the results obtained are based upon pure culture methods and every precaution has been taken to avoid error as a result of contamination.

It perhaps should be mentioned at this point that it is the intention of the writer to deposit living cultures of most of the forms described with the Centralstelle für Pilzculturen.

Systematic Consideration of the Forms Studied.

As has already been indicated, "Bulbils" must in all instances be regarded as representing imperfect conditions of the higher fungi; and like the members of other more or less clearly defined "form-genera" may be associated with perfect conditions included in wholly unrelated genera of the Ascomycetes and Basidiomycetes. They may, moreover, not only represent conditions of such perfect forms, but may be further associated with one or more additional imperfect forms. There may thus be present in some instances a succession of three or even four distinct reproductive phases which together make up the individual life-cycle.

It has been the aim of the present investigation, therefore, to endeavor not only to obtain further information as to the occurrence, morphology, and development of these comparatively little known structures, but by means of careful and extended work with pure cultures to make some further contribution to our knowledge of their actual relationship in different cases.

Bulbils, as a rule retain their vitality a long time so that they germinate readily after a year or more. Their maximum longevity has not been precisely determined, but in some instances, as in Grandinia and Corticium, they have been germinated after three years. This fact of the extensive longevity of bulbils is of immense importance to the fungus, enabling it to withstand long periods of unfavorable conditions, the perpetuation of the species being thus comparatively well assured.

In arranging the materials available for systematic consideration it has been found most convenient to group the forms under four main divisions, namely: those which are known or supposed to be connected with perfect forms belonging to the Discomycetes; those thought to be connected with Pyrenomycetes; those which appear to be imperfect conditions of Basidiomycetes, and lastly those the actual relationships of which are still undetermined. It has seemed best to consider the last group under a single form-genus, Papulospora, this name having been the first which was applied to bodies of this nature, and the variations in the morphology and development in the different species being such that a separation into more than one form-genus does not seem advisable.

DISCOMYCETOUS FORMS.

Previous investigations have brought to light but two bulbiferous Discomycetes; an unnamed species of Peziza observed by Zukal ('85, '86), and *Lachnea thelebioloides* (A. & S.) Sacc. reported by Morini ('88). To these is added a species of Cubonia now reported for the first time, specimens of which were sent for identification to Professor Elias J. Durand of the University of Missouri, to whom the writer is indebted for the following diagnosis:

Cubonia bulbifera n. sp.

PLATE 1, FIGURES 1–28.

"Plants single or gregarious, often crowded, sessile or narrowed to a stem-like base, turbinate, 3–10 mm. in diameter. Disk cupulate or saucer-shaped, the hymenium pale fawn-color, even when young, but in old specimens wrinkled in a cerebriform manner, externally much darker, becoming almost black with age, smooth or grumous; margin irregularly lacerate-dentate. Consistency subgelatinous, excipulum pseudoparenchymatous throughout, of nearly rounded cells, 20–25 μ

in diameter, the cortical cells blackish, often protruding in groups. Asci clavate, apex rounded, not blue with iodine, 125 × 15 μ. Spores 8, uniseriate, hyaline, smooth, spherical, 12 μ diameter. Paraphyses slender, hyaline, only slightly thickened upward. Mycelium giving rise to numerous rounded, black bulbils, 75–100 μ diameter, composed of rounded cells about 20 μ diameter."

Cultivated on nutrient agar. Found on dog dung from Jamaica, Paestum (Italy), Guatemala and California, and pig dung from Guatemala.

This fungus was first obtained by Dr. Thaxter on dog dung from Jamaica and has been kept growing in pure tube-cultures for twenty years; since then he has found it on the same substratum from Paestum, Italy, and from Guatemala. It was also secured from gross cultures of pig dung and of dead flowers believed to be of the genus Criosanthes from the last named locality, while the writer has found it on gross cultures of dog-dung from Claremont, California, from which a pure culture was obtained in a manner similar to that already described. This was not difficult, since the mycelium grows with great rapidity and the bulbils are produced in abundance. The fungus was grown on a great variety of media until the mature perfect form was obtained. The mycelium grows well on nearly all media, producing numerous dark-colored, almost black, bulbils. The best substratum for producing apothecia is bran, or rat or dog-dung, although they developed quite readily on sweet-potato agar or on Irish potato agar with a little sugar; but it was found that after the fungus had been cultivated for a long time on artificial media, it failed to produce mature apothecia.

On appropriate substrata such as bran, dung, etc. the rate of growth of the mycelium is remarkably rapid. The average of several measurements made of this fungus, grown at the temperature of the laboratory is as follows: 1 cm. in 24 hrs., 2½ cm. in 50 hrs., 3½ cm. in 74 hrs., and 5 cm. in 120 hrs. It is white and somewhat flocculent, and does not grow in a "zonate fashion" like that of the Peziza described by Zukal, but spreads out quite evenly over the surface of the substratum. In older cultures the hyphae become quite large, often over 10 μ in diameter, and densely filled with granular protoplasm, but, as they reach their limit of size, they lose their contents. Frequently when a hypha becomes broken or a portion of it is killed, there seems to be a stimulus for growth at the free end, somewhat similar to that in higher plants which are subjected to wounding. This injury of the hyphae appears to cause a sort of damming up of food material, which

is evident from the sprouting out of several small hyphae, not only from the end but also from the sides near the end of the injured part; and these often twine about each other in such numbers, that it gives the appearance of a broom-like structure.

The bulbils.— Often within forty-eight hours, dark bodies, which eventually become black, may be observed with a hand lens, scattered over the substratum or in it; they are most abundant near the point of inoculation, from this point extending out as the peripheral growth of the mycelium increases thus exhibiting a progressive formation. These black bodies are bulbils which soon become very numerous, forming a blackish crust over the substratum and usually giving the whole culture a black aspect. This is especially true when it is grown on such media as potato agar made very hard with about forty grams of agar to the litre. In such cases the mycelium is quite scanty and procumbent, and the bulbils thus become very conspicuous; while on media like rat dung, where there is an abundance of mycelium produced, they are not so readily seen, since they are usually formed on or in the substratum. In the development of these structures which are produced so abundantly, two or three intercalary cells become enlarged and filled with granular nutrient material, as shown in Figures 11–14, Plate 1. From these cells others are produced by budding, or short branches are formed which surround the primordial cells, and which in turn become enlarged so that eventually there is produced an almost spherical bulbil somewhat flattened, 75–100 μ in diameter, the cells in the center, usually considerably larger, but all filled with protoplasm, without any definite differentiation of cell-contents between internal and external cells. Not infrequently, however, the marginal cells of old bulbils lose their contents, although they retain the dark color in the wall, but this is probably due to age. As a result of the unequal production of marginal cells, the bulbils may vary considerably in size and some become quite irregular in outline. Frequently the bulbils or the primordia of imperfect ones, especially as the cultures become old, heap together and form conspicuous dark elevations scattered over the substratum. These structures eventually assume a yellowish color, probably due partly to fading and partly to the immature bulbils that compose them.

The apothecium.— Occasionally there is found a spiral primordium, as shown in Figure 4, Plate 1, produced on short lateral branches which usually divide dichotomously, sometimes of the second or third order, the ultimate branches of which coil up spirally (Figures 1–4,

Plate 1). Ordinarily there are about one and a half to two turns in the spiral, but occasionally there are as many as four. If a lateral branch fails to divide, as it often does, only one primordium is produced (Figure 4, Plate 1). Frequently after the first dichotomy, one of the branches does not divide again, but coils up immediately, while the other may divide once or twice before coiling (Figures 2–3, Plate 1). Thus, according to the number and regularity of these dichotomous divisions, there may appear one, two, or more primordia which are more or less closely related to each other. Usually, however, the pedicels on which they are formed elongate, and thus they may become separated from each other. When this primordium has made about two turns, sometimes as many as four, small branches are produced from the sides of the coils (Figures 5–6, Plate 1), which at this stage often become separated from each other, as shown in Figure 6. It is, however, a very obscure structure, the further details of which are difficult to follow.

Occasionally on media like potato, more frequently on bran, Spanish chestnuts, sweet potato, etc., and quite freely on rat and dog dung, little white patches of hyphae are seen scattered over the substratum. These are the young apothecia. The fine, white, wool-like hyphae become thickly matted together and form a white superficial dome-shaped structure with fine filaments growing out on all sides (Figure 7, Plate 1), and as these become older, they lose their contents and assume a brownish color. Shortly a circular opening appears at the apex (Figure 8, Plate 1), apparently due to the rapid and extensive growth of the inner portion of the apothecium. This opening gradually increases in size, often exhibiting a conical depression in the center which, as the hymenium enlarges, becomes flat and then slightly convex. Microtome sections, made at the time of the opening of the apothecium or shortly before, show the upper region closely crowded with long narrow paraphyses, nearly uniform in thickness, which a little later, slightly enlarge at the ends, forming the somewhat even surface of the hymenium (Figures 9–10, Plate 1).

A short distance below the center of the apothecium, when about the age of that represented in Figure 8, Plate 1, a large cell containing deeply staining material is seen in microtome sections. This appears to be the ascogonium and from it very narrow hyphae, which also stain deeply, grow up between the sterile cells of the apothecium, and eventually produce the asci. At maturity the apothecium is brownish, measuring 3–10 mm. in diameter and 3–5 mm. in height; often in groups and occasionally with a short stem-like base

When a portion of the hymenium containing some of the large cells below the sub-hymenium was put in a sterilized Van Tieghem cell in an endeavor to induce the ascospores to germinate, it was found that frequently these large cells, which measure 20–25 μ in diameter, sent out germ tubes, or turned brown, secreted thick walls about themselves and resembled considerably chlamydospores. Figures 26, 27.

Germination of the ascospores. The mature asci are quite uniform, clavate, with the apex rounded, opening by a lid, 125 μ in length and 15 μ in diameter at the widest place. The ascospores are hyaline, spherical, 12 μ in diameter, and arranged in a single row. At maturity all the spores from each ascus are ejected with considerable force blowing off the lid at the apex in a manner somewhat similar to that of Ascobolus, and thus are thrown in a launch for several centimeters, and, by means of the protoplasmic material which surrounds them, adhere readily to any glass surface with which they may come in contact. These spores were allowed to strike a sterilized cover glass and then supplied with nutrient material and cultivated in a Van Tieghem cell, which had previously been thoroughly sterilized. Not only were the spores alone used as just stated, but frequently a portion of the hymenium with the asci was gouged out with a sterilized platinum needle and hanging drops made of it. In an effort to get these spores to germinate, various kinds of media were used, such as — potato, prunes, bran, horse dung, dog dung, Spanish chestnuts, carrots, etc., either as a decoction, or more often solidified with agar. In spite of these varied efforts, the spores could not be made to germinate. The writer some time ago succeeded in getting the spores of Ascobolus to germinate in Van Tieghem cells by first crushing them lightly between two glass slides, and it occurred to him that the same method might be successful here also. Accordingly hanging drops were made as before, using different media, but the spores were first crushed with a sterilized platinum spatula on the cover-glass. This method proved successful. These spores are composed of a thick brittle episporium and a thin flexible endosporium; the object in crushing was to break the former without injuring the latter. Many of the spores thus crushed were totally destroyed, and broken portions of the episporium were scattered over the culture; but in a few cases, where the pressure was sufficient just to break the episporium without injuring the endosporium, it was found that germination took place in from 24 to 48 hours. Figures 22–24, Plate 1. When this occurs the endospore pushes out, forming a germ tube which is

only a little smaller in diameter than that of the spore itself (Figure 22), and frequently when it has grown a short distance, broadens out as much as 14 μ in diameter (Figure 23). Thus the primary hypha from the ascospore is very large (7–14 μ in diameter), well filled with food material, and grows quite rapidly under favorable conditions. The culture of these germinating spores was carried on in Van Tieghem cells until bulbils were produced on the mycelium.

Germination of the Bulbil.—The bulbils, unlike the ascospores, germinate with great readiness within twenty-four hours and any of the cells that contain protoplasmic material may send out a germ tube, which shortly produces other bulbils from intercalary cells, as described above. When the bulbils are crushed, the contents of each of the large cells escapes surrounded by an endosporium (Figure 19) and germinates readily in Van Tieghem cells. Little significance can be attached to this fact, however, as not only are nearly all bulbils similar in this respect, but it is a common occurrence among spores which are surrounded by a thick episporium, such as the ascospores just considered.

In prolonged cultures of this fungus no other spore forms have been observed.

Lachnea thelleboloides (A. & S.) Sacc.

The association of this species with bulbil-like bodies is reported by Morini ('88) but it is not clear from his account whether the structures seen were true bulbils, or abortive apothecia, as he believed them to be. The apothecia, which he describes and figures, are very similar to those of *Cubonia bulbifera* but the spherical spores of the latter distinguish it at once.

The bulbil-like structures which he describes were found only in old cultures in which the nutriment was more or less exhausted, and are described as irregularly globose, 160–220 μ, and rather hard. In many cases large cells of somewhat spiral form were visible in these bodies which Morini considered "rudimentary ascogonia." The protoplasm of the external cells, is said to be replaced by an aqueous liquid and the walls become thick and brownish-red in color. A large number of the superficial cells, as in the case of the developing apothecium, give rise to short, often septate setae, which cover nearly the whole surface. When these "bulbils" were transferred to fresh substrata, only those with better developed "ascogonia" continued their development until they formed apothecia identical in character with those produced normally. In all other cases,

especially those in which the so called "ascogonium" had completely disappeared, Morini observed no further development, except that in rare cases, a few paraphyses were found.

He is of the opinion that these "bulbil-like" bodies are degenerate apothecia, analogous to the bulbils of Eidam, Karsten, etc., and concludes his article by saying that "the forms heretofore called 'bulbils' or 'spore-bulbils' are to be considered as exactly homologous to apothecia of which they represent forms more or less degenerate or modified during many generations of unfavorable conditions."

PEZIZA, species; not determined.

A species of "Peziza" found by Zukal growing on a laboratory culture may be here referred to, which according to his account is associated with small bulbils 30–40 μ in diameter, reddish brown in color, and produced by "two or three small hyphal branches which wind about one another like serpents or twist, screw-like." The primordium of the apothecium is somewhat vaguely described. The ascospores are said to be elliptical, hyaline, smooth, about $9 \times 6\ \mu$, obliquely monostichous, germinating readily in from twenty-four to thirty-six hours. Since this form does not appear to have been studied by means of pure cultures its connection with the bulbils described must be regarded as somewhat doubtful.

PYRENOMYCETOUS FORMS.

In the review of the literature a number of pyrenomycetous forms that produce bulbils were mentioned, which have been referred either to the genus Melanospora or to the allied genera Sphaeroderma or Ceratostoma. More than twenty different gross cultures made by the writer of various substrata, such as onions, straw of various kinds, paper, pasteboard, Live Oak chips, rotten planks, tubers of Dahlia, old leather gloves, etc., have produced bulbils which in pure cultures have yielded melanosporous perithecia. In a few cases the perithecial form was found on the original substratum and cultures were made from the cirri of discharged ascospores, which on nutrient agar produced bulbils.

In addition to bulbils, all of these forms also produce ovoid, hyaline conidia borne on characteristic bottled-shaped sterigmata. The ascospores are yellowish brown, becoming black or smoke-colored, asymmetrical, more or less crescent shaped. They vary but little

in size, the measurements of *Melanospora papillata* and *M. cervicula* averaging $10 \times 25\ \mu$ while those of *M. anomala* are slightly larger, $12 \times 28\ \mu$. These variations, however, are so small that they could not alone be considered as specific. The size and shape of the ascospores also correspond quite closely with those of *Melanospora Gibelliana* and *Sphaeroderma bulbilliferum*. At maturity the ascospores appear as an irregular black mass in the center of the perithecium. As in all the species of Melanospora the asci are very evanescent. The walls become gelatinous and swell by the absorption of water, which increases the volume to such an extent that the mucilaginous mass protrudes from the ostiole, carrying out with it the embedded spores. If the atmosphere is somewhat humid, this mass of spores, as they are forced out, aggregate in a spherical mass at the mouth of the ostiole; but if the air is dry as they are pushed out, they adhere together into a long, twisted, tendril-like filament, something like the paint as it is squeezed out of an artist's paint-tube. These cirrose structures may measure from 10–18 mm. in length, and twist up into a variety of shapes. The spores not infrequently germinate while still in the cirrus, giving it a white appearance.

Microtome sections show no paraphyses between the asci, but from the walls there grow out more or less conspicuously into the cavity above the asci, numerous hyphal branches, as paraphyses, which converge radially and extend upwards towards the ostiole. These probably aid in the formation of the neck when it is present.

In general the culture methods used were the same for all. Gross cultures of the various substrata were made in crystallizing dishes which were half-filled with sphagnum and covered with white filter paper, on which the substratum was placed. The whole was then well supplied with water and covered with a piece of plain glass and set in a place in the laboratory where it would be protected from the direct sunlight. When bulbils were observed, individual ones were carefully picked out under a dissecting microscope and cultures made from them, until a pure culture was obtained. These were grown on various kinds of media until perithecia with the characteristic long cirri of ascospores, were obtained. Transfers of the ascospores were then made by touching one of the aerial cirri with a piece of nutrient agar on the end of a sterilized needle. In all cases pure cultures of ascopores obtained in this way produced bulbils.

The germination of the ascopores was followed in Van Tieghem cells until bulbils were again produced on the mycelium, thus demonstrating the connection between the ascospore and the bulbil.

In these forms the very young perithecium can be readily distinguished from the bulbil, not only by its mode of development when that is different, but also by the color. The bulbils turn brownish at a very early stage in their development, such as is represented, for example, in Figure 2, Plate 2, while on the other hand, the perithecia frequently remain colorless, or nearly so, until they are beyond the size of the average mature bulbil, and the ascogonium usually can be distinctly seen in the form of one or two large cells lying towards one side of the young perithecium.

The question of sexuality in connection with the formation of the ascogenous primordia has not been worked out. Structures have been observed that might well be taken for antheridial branches, but their attachment was not constantly or certainly observed, so that this phase of the problem will have to be left for future consideration.

Among the twenty bulbil cultures from different sources which have been found by the writer to produce melanosporous perithecia, at least three distinct species appear to be distinguishable. Although these forms possess ascospores that show little if any variation, the differences in their perithecia, bulbils and secondary spore-forms are such that they cannot be included in a single species. Moreover, the characteristics are believed to be sufficiently distinctive to warrant their consideration as separate species. They have therefore been named *Melanospora papillata*, *M. cerricula*, and *M. anomala*. There thus appear to be several closely related Melanospora-like forms, including *Sphaeroderma bulbilliferum*, *Melanospora Gibelliana* and *M. globosa* all of which give rise to bulbils.

The differences which distinguish the perithecia of these forms may be summarized as follows:

Melanospora Gibelliana; neck of perithecium long and tapering, with terminal setae, asymmetrical ascospores.

M. globosa; neck of perithecium longer than *M. Gibelliana*, no well-defined terminal setae, symmetrical ascospores.

M. papillata, n. sp.; perithecium with a distinct papilla only with terminal setae, asymmetrical ascospores.

M. cerricula, n. sp.; perithecium with a short neck, terminal and lateral setae, asymmetrical ascospores.

M. anomala, n. sp.; perithecium more or less definitely papillate, with occasional indications of abortive terminal setae, asymmetrical ascospores.

Sphaeroderma bulbilliferum; perithecia without papillae or setae.

The species of "Sphaeria" mentioned by Biffen as associated with *Aerospeira mirabilis* and the species of "Ceratostoma" connected

with bulbils by Bainier may also be melanosporous and will be referred to later on.

Melanospora papillata n. sp.

PLATE 2. FIGURES 1–26.

Perithecia scattered or gregarious, superficial, membranous, semitranslucent, straw-colored to light brown, globose to pyriform, 350–450 $\mu \times 400$–500 μ, papilla surmounted by erect, somewhat divergent, continuous setae, 100–170 μ in length; primordium a group of one or more intercalary cells; ascospores asymmetrical, somewhat crescent-shaped 10×25 μ, yellowish brown becoming black; conidia abundant, hyaline, spherical to ovoid, on flask-shaped sterigmata; bulbils yellowish brown, irregular in outline, 50–60 μ in diameter, sometimes considerably more than this.

On Live Oak bark (*Quercus agrifolia* Née) from Pomona, California.

A pure culture of this species was easily obtained by making a transfer of the ascospores in the manner already described, on rich nutrients, fairly soft, with about 20 gm. of agar to the litre, and both perithecia and bulbils were produced abundantly. On substrata, however, poorly supplied with nutrient material, such as sterilized agar-agar, or even on a medium well supplied with food material if made very hard (about 40–50 gm. of agar to the litre) the bulbils are very sparingly produced if at all, the mycelium is quite inconspicuous and the perithecia appear scattered over the surface more or less abundantly. In its capacity to retain its power of producing perithecia this species resembles *M. carriendo*, while it is in sharp contrast to some other melanosporous forms studied in which, after long artificial cultivation the bulbils tend to become the dominant mode of reproduction and the perithecia are produced sparingly if at all.

The bulbils. The hyphae, which vary in diameter from 1–7 μ, are hyaline, with numerous oil globules and prominent cross walls, and are usually very scantily developed. The bulbils make their appearance as small straw-colored bodies scattered somewhat sparingly and usually in small patches over the surface of the substratum. In the process of development, which was carefully followed in Van Tieghem cells and in pure cultures in test-tubes, hyphae divide up into short intercalary almost isodiametric cells, one or more of which enlarge (Figure 1, Plate 2) while the contents becomes densely granular and filled with oil globules. At this stage these enlarged cells are

colorless or opalescent with a comparatively thick wall and look much like chlamydospores. The adjacent cells of the filament on either side of them become stimulated and also enlarge to some extent, but remain colorless longer than the others, although they are eventually incorporated into the bulbil. The primordial cell or cells soon become brownish and produce others by gemmation, which in turn produce still others (Figures 2–5, Plate 2), so that the mature bulbil finally consists of one or two, occasionally more, large central cells with slightly thickened walls, surrounded by a number of smaller less highly colored ones, with thinner walls. The mature bulbils measure from 50–60 μ in diameter, although they may vary considerably.

Sometimes three or four intercalary cells enlarge and take part in this process, producing an elongated, somewhat irregular bulbil, while at other times there are as many as eight or ten such cells; but in this latter case they seldom go farther than the production of a few lateral cells which soon become empty and colorless, as is shown in Figure 7, Plate 2.

Not infrequently the terminal cell or series of terminal cells becomes the primordium (Figures 24–25), the further development of which is the same as the one already described. In Van Tieghem cell-cultures, bulbils are sometimes produced with more central cells than ordinarily occur in tube-cultures, and these, which are usually spherical, contain oil globules which give them a peculiar, somewhat opalescent appearance. The cortical cells in such cases are somewhat flattened, as indicated in Figure 22, Plate 2, a condition which may be due to the pressure exerted by the increased number of the central cells.

The perithecium.— The form of the primordium of the perithecium is essentially the same as that of the bulbil but the former, as has already been stated, can, even in the early stages of its formation, be readily distinguished from the latter by the fact that it is colorless. It can be distinguished also from the primordium of the perithecium of *M. cervicula*, which in many respects it resembles, by the fact that the latter turns brownish at a much earlier stage in its development, producing a large number of radiating hyphae, so that its outline is soon indistinguishable.

Usually one, rarely two, intercalary cells take part in its formation, and from these, two or three large cells are produced laterally by budding (Figure 8, Plate 2). From the intercalary cells, or, more frequently, from the adjacent ones of the hypha, branches are sent

up which eventually enclose this group of large cells. These branches which divide up into short cells, form the wall of the perithecium.

Sometimes, as in the case of the bulbil, a terminal cell may become the primordium, as is evidently the case in Figure 10, Plate 2, where there are two large cells which have originated from a terminal one.

The mature perithecium is straw-colored, globose or slightly pyriform, measuring 400–500 μ in diameter, but often much smaller than this, the variations in size are largely due to the character of the medium on which it grows. It is surmounted by a crown of setae which surround the ostiole and are colorless, 100–170 μ in length, stiff, erect, straight, and tapering to a point. There are no lateral setae of this nature, but frequently superficial cells near the base of the perithecium may send out filaments which serve as attachments to the substratum. The perithecia often occur grouped in considerable numbers and not infrequently two or three are found which have more or less fused during their development, having no doubt arisen from primordia which were in close contact with each other. Some time after their formation the cirri of ascospores begin to assume a whitish appearance which is due to the presence of numerous germinating spores producing many abnormalities. A very common form in such cases is shown in Figure 14, Plate 2 where, instead of a regular germ tube, a large opalescent, spherical body is formed at the end of the spore, which contains a great deal of granular material and stains deeply. Occasionally a second such body is produced, and from these one or more lateral branches may arise (Figures 18–20, Plate 2). Not infrequently a series of these swollen cells appears terminating a branch and these become spherical and form a bulbil-like structure (Figure 17) such as is sometimes met with in Van Tieghem cell cultures (Figure 21). One of the most striking features of these germinations is the copious formation on the germ tubes of ovoid conidia which arise from bottle-shaped sterigmata and usually adhere in short chains, although they sometimes cohere at the tips of the sterigmata in a spherical mass. As already mentioned conidia similar to these are also quite frequently met with on the mycelium in all parts of the culture, and when the spores collect in masses the fructification might readily be mistaken for that of Hyalopus.

In some cases the outer cells of the bulbils increase in numbers until the whole structure is about half the size of a perithecium, although very irregular and sclerotium-like. In each case, however, the cells of the original bulbil retain their deep tan-color, while those which have resulted from this secondary growth are distinguished

by light colored walls resembling those of the typical perithecium. The occurrence of such abnormal forms, which may be quite frequently produced on media rich in nutriment such as bran-agar for example, and their resemblance to young perithecia, suggested the possibility of a direct development of perithecia from bulbils similar to that suggested by Bainier ('07), and an effort was accordingly made to determine this point. Individual bulbils showing this tendency were isolated and their further development watched in Van Tieghem cells, while others were transferred to different kinds of media, moist cotton, moist filter paper, etc., but in no instance could they be induced to develop into perithecia, although when the moisture was sufficient, they produced numerous germ tubes which grew out forming the typical mycelium.

Melanospora cervicula, n. sp.

PLATE 3, FIGURES 16–24.

Perithecia scattered or gregarious, superficial, membranous, semi-transparent, straw-colored to brownish, globose to pyriform, 350–450 × 450–550 μ, with a definite neck 85–140μ in length, terminal setae 100–170 μ in length, erect, somewhat divergent, continuous, sharp, subulate; lateral setae on the neck and upper part of the perithecium; ascospores asymmetrical, somewhat crescent-shaped 10 × 25 μ, yellowish brown becoming black; conidia common in tube cultures, hyaline, spherical to ovoid, on flask-shaped sterigmata; bulbils yellowish brown, irregular, normally 50–60 μ in diameter, sometimes 100 μ, primordium one or more intercalary cells. This form is also said to produce conidia on secondary "Harzia-like" heads, and chlamydospores resembling those of *Aeremoniella atra*.

On rabbit dung, Cambridge, Mass.

This melanosporous fungus was obtained from Dr. Thaxter who had grown it for some time as a pure culture. It was originally found on a gross culture of rabbit dung from the vicinity of Cambridge, Mass., and has proved to be of special interest on account of its different methods of reproduction.

In addition to perithecia and bulbils, this fungus seems to have associated with it two other spore forms, chlamydospores resembling those of *Aeremoniella atra* and also conidia produced on secondary heads resembling those of the genus Harzia. Alcoholic material furnished by Dr. Thaxter was used for the study of these two modes of reproduction. This material was the result of a series of transfers

of the cirri of ascospores and therefore probably pure. The writer has under cultivation transfers of this same fungus but although it has been grown on various kinds of media, both very rich and very poor in nutrient material, and hard and soft, etc., yet thus far he has not succeeded in obtaining either the chlamydospores or the "Harzia-like" fructification. This is probably due to the fact that the production of these structures is secured under certain peculiar conditions not readily controlled.

In general this fungus resembles *M. papillata* in form and habit of growth. The predominant type of reproduction in both is by ascospores the production of bulbils being scanty, while in some cases, as on attenuated agar cultures, they are not produced at all. The perithecium of *M. cerricola* which is usually 400–500 μ in diameter, has a definite neck 85–140 μ in length, while *M. papillata* which is slightly smaller, seldom reaching 500 μ in diameter, has no neck but often a papilla-like structure from which the setae arise. Moreover, the former probably produces conidiophores of the "Harzia type" and also chlamydospores which resemble those of *Neeromonilla atra*.

The Bulbils.—The mycelium is colorless, procumbent or only slightly aerial, growing evenly over the surface of the substratum. The hyphae, which are copiously septate, measure 5–7 μ in diameter, but often large swellings occur in them which seem to act as storage organs and from which several branches may grow out as shown in Figure 21, Plate 3. These are found not infrequently on attenuated artificial media such as agar alone without any nutriment, on which the mycelium is very scanty, being barely visible even with the aid of a hand lens. On such media, it should also be noted that as in *M. papillata*, bulbils are not produced. It further resembles the latter in the mode of development of the bulbils, the primordium consisting of a group of intercalary cells. It is, however, subject to considerably greater variation and many irregular, incomplete or imperfect forms appear. Since the mode of development is essentially the same as that described for *M. papillata*, it will be unnecessary to repeat the description here. They are, however, produced very sparingly on most media, and on some, such as that just mentioned, do not occur at all, although on a rich substratum not too hard, such as sugar, chestnut or bran agar they are produced quite abundantly.

The perithecium.—In general the perithecium resembles that of *M. papillata*, but is clearly distinguished by having a definite neck. They, however, vary considerably in size, sometimes reaching 550 μ in diameter, their form often being somewhat contorted, with only

a slight difference in size between the neck and body, while at other times several may be grown together. The neck is short, 85–140 μ in length surmounted by a group of terminal setae of about 100–170 μ in length. The mode of development of the perithecia is somewhat variable. Although at times they seem to be produced from intercalary cells, yet more frequently a short lateral branch is produced which may form a close coil of one or two turns, and occasionally even a definite spiral is found as is shown in Figure 19, Plate 3. The young perithecia turn brownish at a much earlier stage of their development than either those of *M. papillata* or *M. anomala*. This fact, together with the large number of radiating hyphae that are produced from the initial cells, a condition not occurring in either species just mentioned, make it very difficult to follow the early development. When the perithecium is young before the neck is produced, filaments with thick brownish walls, apparently stiff and with prominent septa, are seen scattered sparingly over the surface and radiating from it. They are formed by the outgrowth of some of the peripheral cells, and as the perithecium becomes older, as has already been stated, their number increases and some grow down into the substratum and act as hold fasts.

The "Harzia-type" of reproduction.— This mode of reproduction which was studied from material preserved in alcohol appears in small tufts scattered over the surface of the substratum. Short lateral branches become swollen at the end after the fashion of Oedocephalum or Aspergillus, and from this head a number of flask-shaped sterigmata are produced, on the ends of which occur secondary heads crowded with hyaline conidia which are usually spherical and sessile but occasionally more or less ovoid and furnished with short stalks (Figure 24, Plate 3). The secondary heads seem to vary considerably in size, and being so completely covered with conidia it was difficult to determine at all times the exact relation of the different parts of this fructification. In several cases there appeared to be little or no swelling of the secondary head, but with the limited amount of material at hand this could not be determined with certainty. Occasionally the head instead of being spherical is somewhat elongated, and the bottle shaped stalks, on which the secondary heads are formed, are scattered along the margin of this as shown in Figure 23, Plate 3. This fungus also produces numerous spherical conidia on bottle-shaped sterigmata along the margin of the hyphae, similar to those described for the other melanosporous forms.

The chlamydospores.— On the preserved material already referred

to, there were also found associated with the "Harzia-like" fructification, chlamydospores which are ovoid, smooth, brownish, thick-walled, and have the distal end rounded. They are produced usually on short lateral branches which taper towards the tips and may be continuous or septate. The mature spores are quite uniform in size, about 17 × 21 μ, although there were some that appeared to be mature, which were slightly smaller than this. These spores resemble both in color and form those of *Acremoniella atra* Sacc. There are certain other fungi that produce imperfect forms of the "Harzia" and "Acremoniella" type which will be further considered below in connection with *P. aspergilliformis*.

Melanospora anomala n. sp.

PLATE 2, FIGURES 27–30; PLATE 3, FIGURES 1–15.

Perithecia scattered or gregarious, superficial membranous, straw-colored or light brown, globose or subglobose, 250–350 μ × 350–450 μ, ostiole formed in connection with a definite but inconspicuous papilla without setae, primordium a spiral of 4 or 5 coils; ascospores asymmetrical, somewhat crescent-shaped 14 × 28 μ, yellowish brown becoming brownish black; conidia, hyaline, spherical to ovoid, on flask-shaped sterigmata; bulbils yellowish brown, variable in size 70–140 μ in diameter, sometimes elongated ones 180 μ in length, primordium a group of intercalary cells.

On Spanish chestnuts in laboratory culture.

Gross cultures of Spanish chestnuts, which were imported probably from Spain obtained by the writer in the Boston market, produced numerous brownish colored bulbils when cultivated in moist chambers. By using the general methods already described, separate bulbils were transferred to sterilized nutrient-agar tubes and, after a few transfers, were obtained pure.

The mycelium of this fungus is white and more or less aerial, varying according to the media in which it is grown. When grown on soft chestnut agar, it becomes quite flocculent, while on chestnut decoction it forms a more or less felted layer over the surface, assuming the brownish color of the liquid; but on potato agar its growth is rather scanty. The diameter of the hyphae varies from 2.5–7 μ.

The bulbils. Scattered over the aerial hyphae and on the substratum are seen numerous small yellowish-brown bulbils, which, when examined microscopically, are found to vary considerably in size and outline, many of them nearly spherical, others somewhat elongated.

Usually there is no differentiation between the cortical and central cells, but in old bulbils several empty cells, which may or may not be colorless, are often found loosely attached to the periphery. The central cells are often larger than the more superficial ones, but this is not always true, since in many instances they are perfectly uniform throughout. These bulbils are usually developed from a lateral branch which divides up into short cells. These produce short secondary branches (Figures 27, 29, 30, Plate 2) which also divide up into short cells and may produce others by a process of gemmation. Sometimes the primordium consists of a group of intercalary cells (Figures 28, Plate 2, and Figure 11, Plate 3) which may produce other cells by budding in a manner somewhat similar to that of *M. papillata*. At maturity the bulbils are irregularly spherical, about 70–140 μ in diameter, but where several intercalary cells have taken part in its formation, the long axis frequently measures 180 μ. This bulbil may be distinguished from *M. papillata* or *M. cerricola* by the fact that the cells are usually homogeneous throughout, while in the latter two there is a more or less definite cortex. The margin is also often more irregular in the bulbil under consideration as is shown in Figure 15, Plate 3. In the immature bulbils which show this uneven outline more markedly than the mature ones do, there sometimes appear short branches of two or three seriate cells which extend beyond the others.

The perithecium.— In an effort to induce this fungus to produce the perfect form, it was grown on various kinds of media. Decoctions of potato, bran, corn meal, Spanish chestnuts, etc., were hardened with agar-agar, some hard, some soft, but nothing except variations in the size and development of the bulbil could be obtained. Finally, after removing the shells of some fresh, sound chestnuts, the kernels were sliced up and used for cultures. On this medium perithecia were produced in abundance. These are almost spherical in form and vary from 300–400 μ in diameter, no ostiole being developed until they are nearly mature, at which time a few cells about the opening form a definite, though inconspicuous papilla. Terminal setae are wholly absent, and only rarely do the superficial cells produce lateral filaments. Frequently, however, short projections are observed from some of the cells that compose the papilla, as if an attempt were being made to produce setae. The perithecia are light yellowish-brown in color, much lighter than that of the bulbils, and so translucent that the spores can be readily seen grouped together in a black mass in the center (Figure 12, Plate 3).

Development of the perithecium.— The primordium of the perithecium is quite different from that of the bulbil. In this case a short lateral branch coils up spirally, usually making about four or five turns, but in some cases as many as eight. Figures 1 to 8, Plate 3, represent successive stages in the development of the spiral. Usually the second and part of the the third turn become enlarged while branches are given off from the first or from the cells below it. These branches grow up around the spiral and often send secondary branches in between the swollen lower coils so that they are forced apart (Figures 7, Plate 3). The branches continue to grow until they have enveloped the whole spiral, which soon loses its characteristic form. It would appear that the upper portion of the spiral either becomes a disorganized mass of mucilaginous material or not infrequently seems to be pinched off and ejected during the formation of the wall of the young perithecium, as is shown in Figure 7, Plate 3. By the time the wall is completed all that can be recognized of the spiral are two or three large cells which come to lie free in a cavity usually towards one side of the perithecium and which stain deeply (Figures 9–10, Plate 3). Sometimes branches seem to come off from each of the coils, so that one finds the spiral with a number of very short lateral branches produced from its outer surface. Occasionally also the lateral branch that produces the spiral, while making its first coil, divides into short cells and sends off secondary branches from these, as shown in Figure 3, Plate 3. Whether either or both of these develop into perithecia or bulbils, or are to be regarded as abnormalities, could not be determined, since they were of rare occurrence.

Conidia on bottle-shaped sterigmata, similar to those produced by *M. papillata* also occur in this species (Figure 13, Plate 3). Germinating ascospores particularly, produce them abundantly in a dry atmosphere, but they are more sparingly developed on the mycelium.

This fungus resembles somewhat a form described by Berlese ('92) under the name of *Sphaeroderma bulbilliferum*, which is referred to below. The former has, however, a slightly smaller perithecium (300–400 μ in diameter) with a papilla about the ostiole, while the latter is 400–500 μ in diameter, and has no papilla, the ostiole being flush with the surface. The Sphaeroderma moreover is said to have connected with it large two-celled chlamydospores, which have not been found associated with *M. anomala* although the writer has repeatedly searched for them. Berlese does not describe the method of development of the bulbils, but states that "the sporeballs resemble those described by Mattirolo as belonging to *Melanospora Gibelliana*."

The bulbils of the latter are not unlike those of *M. anomala* in size, color and mode of development.

The species of "Sphaeria," referred to by Bilfen ('02) in connection with *Aerospora mirabilis*, also resembles somewhat *M. anomala*. It differs from the latter, however, in several important respects. The perithecium has no papilla about the ostiole, the ascospores are symmetrical and the primordium of the bulbil is a spiral.

Again the mode of development of the perithecium from a spiral primordium resembles somewhat that of *Melanospora stysanophora* described and figured by Mattirolo ('86). The mature perithecia however, are different, *M. stysanophora* having a distinct neck. The latter is also said to be associated with a Stysanus-like fructification.

Melanospora Gibelliana Mattirolo.

This species was found by Mattirolo on a gross culture of decayed chestnuts in moist sand, and besides melanosporous perithecia and bulbils it also produced chlamydospores and conidia on bottle-shaped sterigmata.

The perithecium, which develops from a spiral primordium, is somewhat pyriform with a long neck surmounted by terminal setae. The neck, however, is considerably longer than that described for *M. cerricola*. The ascospores are brownish-black and asymmetrical, somewhat similar to those described for the other melanosporous forms.

The bulbils are said to be nearly spherical, pale yellow to brownish-yellow, and often 100 μ in diameter, with a colorless cortical layer of cells resembling somewhat the appearance of *Papulospora coprophila*. In its development a short lateral branch divides and forms a number of short secondary branches which intertwine forming an irregular spherical body varying considerably in size.

This species also is said to have associated with it chlamydospores somewhat resembling Sepedonium, as well as conidia on bottle-shaped sterigmata.

Melanospora globosa Berl.

In the same article in which he describes *Sphaeroderma bulbilliferum* ('92) Berlese also describes *Melanospora globosa* which he found growing on small pieces of decaying wood and herbaceous material. The perithecium of this species is, as the name indicates, globose, 250–280 μ in diameter and 360–450 μ (rarely 500 μ) long. The neck

is well developed, 110–200 μ in length. The ascospores differ from those, already described, in being symmetrical. The other forms have asymmetrical ascospores which are somewhat crescent-shaped.

Besides the perfect form this species is said to have: microconidia which resemble those of Acrostalagmus; chlamydospores that are of the type of *Acremoniella atra;* and bulbils which he considers of the same nature as similar structures described by Mattirolo. Berlese succeeded in obtaining bulbils on the mycelium produced from ascospores but he failed to find any perfectly developed.

Sphaeroderma bulbilliferum Berl.

This species which is described by Berlese ('92) was found growing on dead leaves of Vitis, Cissus, and Ampelopsis. It is said to have several kinds of reproductive bodies, such as ascospores, bulbils, conidia and chlamydospores.

The perithecium is globose or sub-globose, 400–500 μ in diameter, without any neck, setae or papilla. These characteristics distinguish it from any of the melanosporous forms already referred to. It resembles *M. anomala* but is slightly larger and has no papilla. The ascospores are brownish-black and asymmetrical.

The bulbils are yellowish, nearly spherical, 80–150 μ in diameter, consisting of polyhedral cells and surrounded by a layer of empty cortical cells. They are said to resemble quite closely those described in connection with *Melanospora Gibelliana.*

The conidia occur in chains on bottle-shaped sterigmata resembling those of the melanosporous forms already referred to.

The chlamydospores, which measure 32–40 × 24–25 μ, are described as yellow, oval, smooth, composed of two unequal cells, and formed terminally on the ends of short lateral branches.

"Ceratostoma" sp. indet.

Bainier ('07) has reported that he has determined the connection of a perithecium of the genus Ceratostoma with *Papulospora aspergilliformis.* He is of the opinion that the bulbils in this instance are immature perithecia and that, under proper conditions as regards nutriment and moisture, they may be induced to complete their development.

In this form, the bulbil is produced by a short lateral branch which coils up spirally, the coils becoming quite compact. One or more of the terminal cells enlarge and eventually become filled with

conspicuous food material. The cells below the spiral send out branches which divide and may, in turn, produce others. These grow up around the spiral and completely envelop it, thus forming a somewhat spherical mass of cells. In a moist atmosphere these are said to develop into sclerotium-like bodies. By transferring these large bulbils to pieces of moist bread, Bainier succeeded in inducing them to develop into perithecia which he refers to the genus Ceratostoma, although it is not evident why this form should not also be referred to Melanospora. This subject will be further dealt with below under *Papulospora aspergilliformis*.

In connection with pyrenomycetous forms it will be well to consider briefly two additional species which may be regarded as doubtfully pyrenomycetous.

FORMS DOUBTFULLY REFERRED TO PYRENOMYCETES.

Papulospora candida Sacc., parasitic on Geoglossum, has been reported by Dr. Thaxter to be connected with hypocreaceous perithecia found on specimens of the host obtained in South Carolina; but this material was, unfortunately, not available for examination, and since pure cultures of this fungus grown on different media have thus far failed to produce any perfect form, its position must, for the present at least, remain more or less uncertain. The fact, however, that the bulbil is definitely connected with a Verticillium would seem to afford strong evidence of its hypocreaceous nature. A second doubtful form is *Acrospeira mirabilis* (Berk. & Br.), with which Biffen ('02) has associated a species of "Sphaeria," but since he was unable to obtain the bulbils or "chlamydospores" as he terms them, of Acrospeira from pure cultures of the ascospores, his conclusions must be accepted with some reserve.

Papulospora candida Sacc.

Plate 4, Figures 1–17.

This fungus was first found by Ellis who collected it in New Jersey and distributed it by N. A. F. No. 3673. The species appears to be common and distributed from N. Carolina to Maine. The material for the present investigation was found growing abundantly as a parasite on *Geoglossum glabrum* in a maple Sphagnum swamp near Walnut Hill, Mass. It was first described (Mich. II, p. 576) as *Papulospora candida*, by Saccardo who also mentions that *Verti-*

cillium agaricinum Link, var. *clavisedum* (Mich. II, p. 577) is associated with it.

A large number of specimens of Geoglossum, with plenty of Sphagnum and leaf mould about each, were collected — some infected, others not — and were grown under bell jars or in a large germinating vessel with a glass top. It was thus kept growing for nearly two months, until it could be determined whether the Papulospora would grow as a saprophyte on artificial media. A number of tube cultures were made of the bulbils on various kinds of media, the most successful of which were the ascoma of Geoglossum itself. About a dozen large specimens of these with long stalks were selected and each put in a test-tube which had previously been supplied with about half an inch agar. These were then sterilized in an autoclave, the object of the agar being simply to hold the specimen in place and thus lessen the chances of contamination in making the transfers, etc. On this medium a pure culture was eventually obtained, which was then transferred to other media such as potato, corn meal, chestnut, horse dung, etc., hardened with agar. This fungus grows fairly well as a saprophyte, better on hard than on soft media such as potato and bran, but very slowly on horse dung, on which, after a month, it had not grown much more than an inch from the point of inoculation. Associated with the Papulospora on the ascoma were found, among other fungi, specimens of *Pleurage anserina* (Rabh) Kuntze and *Verticillium agaricinum* Link, the latter producing in pure cultures very large and conspicuous, brownish sclerotia.

On its natural host *Papulospora candida* forms conspicuous white blotches spreading over the upper portion of the ascoma (Figure 47, Plate 4), and if not too wet, extending down the stem. Although the host is usually found in damp sphagnum swamps, the parasite is largely confined to those specimens that grow tall, so that their tops are comparatively dry. The mycelium is white, procumbent, branching copiously, but soon becoming indistinguishable as such, even with a good hand lens, mainly on account of the large number of bulbils that are formed which give the whole fungus a powdery appearance. When examined under a microscope the mycelium is opalescent, owing to the presence of numerous oil globules (Figures 12, 44, Plate 4) and other colorless material in the cells. The cultures become completely covered with the white powdery bulbils which a little later assume a characteristic cream color.

The bulbils.— During the process of development of the bulbil a short lateral branch divides up into a number of cells and the end

[illegible] although the length may vary from [illegible]. In this respect it differs from [illegible] in which the [illegible] cells are smaller and [illegible] in shape. Both of these [illegible] [illegible]

[illegible]

[illegible]

history of the parasite is known." Before Biffen ('02) examined this species, the only method of reproduction known was by its so-called "chlamydospores" which at maturity consist usually of one large, thick-walled, chocolate-brown, warty cell and three or more colorless cells adhering closely to it. By the use of pure cultures Biffen claims to have succeeded in obtaining not only the "chlamydospores," as described by Berkeley and Broome, but also what he calls "spore balls" (bulbils) and definite peritheeia.

The mycelium of Aerospeira is fine, colorless, procumbent, more or less sparingly developed, and produces large numbers of reproductive bodies, which, in their development and structure, are bulbils rather than "chlamydospores." They are so abundant that the whole surface of a culture, which would otherwise be white, assumes a brownish aspect. The readiness with which these bulbils are produced makes it comparatively easy to trace their development, which, in brief, is as follows: an erect lateral branch usually divides into three secondary branches (Figure 18, Plate 5) each of which coils up much like that of *Papulospora parasitica*, to be considered below. They make about one to one-and-a-half coils and divide into three cells by cross septa. The middle one of these three, as a rule, enlarges rapidly, forming the functional spore (Figure 21, Plate 5) (the central cell of *P. parasitica*), but occasionally the end cell (Figure 20, Plate 5) more rarely the third, is the one that functions in this respect; while the other cells of the coil, ordinarily three or more in number, grow less rapidly and eventually lose their contents, become colorless, and adhere to the side of the large cell. If the marginal cells should increase in number so as to enclose the large cell completely, there would be practically the same condition as exists in *P. parasitica* (Figures 16, 17, Plate 5). In the present form, however, the large cell becomes dark brown in color and develops a thick wall, which eventually becomes warty, and measures 25–30 μ in diameter. Figures 18–23, Plate 5, illustrate the stages in the development of this bulbil. Thus in Aerospeira we have a structure that is only slightly less complex than that seen in *P. parasitica*, a form in which many imperfect bulbils can with difficulty be distinguished from some of those of Aerospeira, their only difference being due to the absence of a warty episporium. These bulbils were grown on various kinds of sterilized nutrient material, and most of the experiments described by Biffen were repeated. The culture conditions were varied with regard to media and other conditions of growth, in many of these experiments, but more bulbils of the same kind were always produced

and never, so far as the writer has observed, have any indications been seen of the development of "spore balls," or perithecia such as have been described by Biffen.

BASIDIOMYCETOUS FORMS.

As has already been mentioned (p. 238), bulbils were first reported among the Basidiomycetes by Lyman ('07), who not only definitely connected one form with *Corticium alutaceum* (Schrader) Bresadola, which is dealt with briefly below, but also refers to two other kinds of bulbils, the mycelia of which have well marked clamp-connections; but basidiosporic fructifications were not produced abundantly enough to allow of their identification. Dr. Lyman has kindly supplied the writer with specimens of these forms for the purpose of comparison, which will be referred to under their respective species.

The methods used here were much the same as those already described, except that more gross cultures of wood were used with different amounts of moisture. The best results were obtained from decoctions of bran in one or two litre Erlenmeyer flasks with pieces of rotten wood that extended considerably above the liquid, so that the mycelium could obtain the degree of moisture that best suited it. In order to keep the pieces of wood in place and thus lessen the chances of contamination a quantity of agar was sometimes put in the bottom of the flasks.

Grandinia crustosa (Pers.) Fr.

Plate 6, Figures 1–10.

Bulbils of this species were obtained from at least ten different sources, mostly on substrata such as rotten chips of Live Oak (*Quercus agrifolia* Née), old canvas, paper, cardboard, etc., from Claremont, California. It has been found also by Dr. Thaxter on gross cultures of rabbit dung from Mass. and on rotten wood from Buenos Ayres, and is probably the same as that referred to by Lyman ('07, p. 166), which was obtained by Mr. A. H. Chivers on a gross culture of bits of wood, paper, etc.

The mycelium, which shows quite marked clamp-connections, is colorless, procumbent, producing numerous white fibrous, rope-like strands of hyphae which radiate conspicuously in all directions from the point of inoculation. The white mycelium, however, soon takes on a light straw-colored aspect, owing to the formation of bul-

bils in large numbers, which gradually become darker as they mature. When grown on nutrient agar in large receptacles like Erlenmeyer flasks, after the mycelium has covered the whole substratum with powdery bulbils, new centers of growth-activity occur at different points on the surface of the culture, and the radiate development of the hyphae and the subsequent formation of bulbils are repeated on the top of those first formed. If the flasks have plenty of nutrient and do not dry up, this process may be repeated two or three times, the amount of mycelium, and consequently the number of bulbils formed, decreasing each time, so that eventually there appears a thick powdery mass with here and there large, white, rope-like strands of hyphae persisting, which is all that can be distinguished of the mycelium.

The bulbils are usually more or less spherical in shape, varying from 52 to 88 μ in diameter, although often exceeding this size, especially when the primordia of two happen to be so close together that their hyphae intertwine, thus forming a large irregular body. The individual cells are large, densely filled with granular material and oil globules, spherical at first; but the central ones soon become angular by pressure, while the marginal ones retain more or less their original form. There is no differentiation of a cortical layer; the cell wall and contents are uniform throughout, except that occasionally some of the peripheral cells which project beyond the others lose their contents, but this is the exception and is probably due to age.

The bulbils. – The hyphae which take part in the formation of the bulbils become enlarged, conspicuous, and more or less contorted on account of the prominence and swollen nature of the clamp-connections, which often occur at short intervals. The lateral branches from these divide up into short cells, so that there comes to be a number of almost spherical hyaline cells with fairly thick walls and filled with granular material and oil globules (Figures 4–9, Plate 6). During the formation of new cells, which are also spherical in shape and produced by budding from the marginal ones, the central cells gradually lose their original form and become angular, as a result of the lateral pressure or resistance offered by the outer cells. When the bulbils are nearly mature, they assume a light straw or "rusty-cinnamon" color. Figure 10, Plate 6, represents a mature bulbil, drawn on the same scale as the other mature forms. This method of development follows very closely that described by Lyman ('07) in connection with *Corticium alutaceum*, considered briefly below.

Formation of basidiospores. – The basidiosporic fructification of

Grandinia has been produced on gross wood cultures of this bulbil and also on test-tube cultures of bran-agar of about 40 gm. of agar to the litre, by three or four of the ten cultures from different sources under cultivation. Preparatory to its formation, the mycelium ceases to produce bulbils and forms a sort of incrustation, chalk-white in color and becoming pustulate by the time the spores are formed, Figure 1, Plate 6. The pustules on examination are found to be made up of more or less thickly interwoven branching hyphae, which have become enlarged and densely filled with granular material and oil globules, the ultimate ramifications of which form the hymenium (Figure 2, Plate 6). The basidia, which form a somewhat loose hymenium, each produce four spores, which are ellipsoidal to oblong in shape, measuring about $4 \times 8\ \mu$. These spores were germinated in Van Tieghem cells and the growth of the mycelium followed until the formation of new bulbils, which were transferred to nutrient agar media, where they produced mycelia and bulbils like the original culture.

On tube cultures this fungus occasionally produces typical sclerotia, which are formed by the massing together of many hyphal branches which remain colorless for some time and thus are easily distinguished from the bulbils. Moreover, they are larger, 400–500 μ in diameter, irregular in shape, somewhat darker in color at maturity, and composed of smaller, compact cells.

Grandinia also produces conidia of the Oidium-type on slender clampless conidiophores, such as are described by Lyman ('07) for *Corticium alutaceum*.

Corticum alutaceum (Schrader) Bresadola.

The bulbils of this species were obtained from Dr. Farlow, who found them on a piece of rotten oak bark collected at Chocorua, N. H. It was comparatively easy to get a pure culture, as the bulbils are produced in large numbers and germinate readily. This form has been carefully compared with specimens of *Corticium alutaceum* obtained from Dr. Lyman and they proved to be the same. The development of the bulbil and the character of the conidia are practically identical with those described for Grandinia and, as these have been well worked out in pure cultures by Lyman ('07), it is not necessary to repeat the results here, a detailed description of which may be obtained by consulting his article, pp. 160 and 196. The mode of development of the bulbils and the character of the conidia, however, have been carefully

verified. Lyman obtained his cultures from the basidiospores collected on old rotten oak logs in the field and pure cultures from these produced bulbils. The writer began his cultures with bulbils, also collected in the field, and, after a great number of unsuccessful attempts, finally succeeded in obtaining a basidiosporic fructification similar to that described by Lyman. This was accomplished by using gross cultures of partly decayed wood in two litre Erlenmeyer flasks with sufficient agar to hold them in place. The mycelium, as usual, produced bulbils profusely on the agar and wood, but after six or eight weeks near the top of the pieces of wood conspicuous patches of white mycelium appeared, which eventually produced the hymenium and basidiospores of *C. alutaceum*.

Papulospora anomala n. sp.

Plate 6. Figures 11–19.

This form, which was obtained from four different localities,— three from the vicinity of Claremont, California, found on Live Oak chips, and one on an old paper from Cambridge, Mass.,— has been grown on a variety of substrata in the hope that it would produce its perfect form, but thus far all these efforts have failed. That it belongs to the Basidiomycetes is shown by its clamp-connections, which, however, are not so prominent as those in the two preceding forms, from which it is further distinguished by the dark brown, opaque, almost black color of the bulbils, the compact nature of their cells, and their mode of development. The mycelium is white, procumbent, scanty, slightly aerial on some substrata, with a large number of conspicuous oil globules, and not infrequently contains swollen intercalary cells, which are also densely filled with food material and probably act as storage organs.

The bulbils.— The primary hyphae are small, seldom more than 3 μ in diameter, and do not produce bulbils; but scattered over the secondary hyphae, which vary greatly in width, often reaching 10 μ and under some abnormal conditions 14 μ, are seen slightly swollen, colorless, intercalary cells, quite different from those mentioned above, about 4 or 5 μ in diameter, sometimes projecting considerably and resembling short stunted branches; at other times the base of a short lateral hypha swells slightly and forms the primordium (Figure 12, Plate 6). From the primordial cell or cells branches are sent out in different directions, the basal cells of which become spherical and in turn may produce other similar branches (Figures 13–15, Plate 6).

The lateral walls of these basal cells adhere firmly to each other and the cells become incorporated into the bulbil.

Figures 11–15, Plate 6, illustrate the early stages in the development, and Figures 14 and 15 show the formation of the spherical cells at the center, around the initial cell or cells, while Figure 16 represents a little later stage, which is composed of small hyaline cells with very indistinct walls and forming almost a spherical body with few, if any, cells projecting beyond the others. About this stage, or usually a little later, it would appear that the bulbils cease to form new cells, or, if any, very few, and that the further increase in its size is chiefly due to the enlargement of the individual cells which compose it and which, up to this period, have been small, hyaline, with indistinct walls. As these cells enlarge, there is quite a strong lateral pressure exerted, which tends to make the walls angular, which in the meantime have become more prominent and gradually assumed a brownish tint, that later becomes a dark brown, almost black. As a result of this mode of development, the bulbil at maturity has a clear-cut, even margin, without any appendages or sharp projections, nearly spherical in form, except where some cells in the process of enlargement increased faster than others or in cases where two primordia were formed close together and their early branches became intertwined, forming an elongated, compound structure. The color, which becomes so deep that even the cell walls cannot be distinguished, may be bleached out by placing them in potassium hydroxide for a few hours. The mature bulbils (Figure 17, Plate 6) vary in size, usually measuring from 125 to 175 μ in diameter, although occasionally some are even larger.

Bulbil "No. 200."

This form was obtained from Dr. G. R. Lyman and was originally found by Dr. G. P. Clinton in the vicinity of Cambridge, Massachusetts, on a fragment of an old newspaper in a field. In general this species resembles Grandinia in the mode of development of the bulbils, the presence of conidia and the clamp-connections of the hyphae. The bulbils, however, are much darker and the mycelium does not form the white, fibrous, radiating strands that are so characteristic of Grandinia.

On gross cultures, especially of wood or horse dung agar, the hyphae mass together in conspicuous papilla-like elevations, which are much more prominent than the fructification of Grandinia. These

elevations are composed of closely compacted basidia-like structures. Unfortunately thus far the writer has observed only a few scattered basidia with basidiospores so that it has been impossible to obtain a specific determination.

BULBILS NOT YET CONNECTED WITH A PERFECT FORM AND INCLUDED IN THE FORM-GENUS PAPULOSPORA.

Key to the Species of Papulospora

I. Primordium intercalary.
  A. Bulbils black. . . . . *P. pannosa* n. sp.
  B. Bulbils yellowish to dark brown.
    1. Bulbils, brownish-yellow, central cells 28–55 μ in diameter. *P. immersa* n. sp.
    2. Bulbils straw-color, central cells 10–20 μ in diameter *P. irregularis* n. sp.
    3. Bulbils dark brown, hyphae with clamp-connections. *P. anomala* n. sp.

II. Primordium one or more lateral branches.
  A. Primordium normally a single lateral branch.
    1. Primordium a spiral.
      a. Cells of bulbil heterogenous, definite cortex
        i. One central cell.
          α Cortex complete . . . . . *P. parasitica.*
          β " incomplete . . . . . *Acrospeira mirabilis.*
        ii. More than one central cell.
          α Spiral in one plane, cortical cells spinulose. *P. spinulosa* n. sp.
          β Spiral in more than one plane, 2–6 central cells.
            (a) Bulbils a dark brown . . . . . *P. coprophila.*
            (b) " brick red . . . . . *P. rubida* n. sp.
      b. Cells of bulbil homogenous.
        i. Bulbils brown 21–36 μ in diam. . . *P. sporotrichoides* n. sp.
        ii " steel gray 21–36 μ in diam. . . *P. cinerea* n. sp.
    2. Primordium not a spiral.
      a. Bulbils large, 100–750 μ in diam. . . *P. aspergilliformis.*
      b. " 30–35 μ in diam. cream color . . . . . *P. candida.*
  B. Primordium two or more lateral branches forming a spherical aggregation of cells at the top . . . . . *P. polyspora* n. sp.

Heretofore fungi producing bulbils have been referred chiefly to the form-genera Papulospora and Helicosporangium, but the characters on which these two have been based are not clearly defined, and as already stated, it does not seem desirable to recognize more than one form-genus. Since Papulospora was the name first employed to represent bodies of this nature, all the fungi that the writer has examined that produce bulbils, the perfect form of which has not been determined, are placed in this form-genus which may be described as follows.

Papulospora.

Mycelium extensive or scanty, flocculent or procumbent, usually white but sometimes dark colored. Reproduction by means of bulbils, i. e., reproductive bodies of more or less definite form, composed of a compact mass of homogeneous or heterogeneous cells which may be few or many, but are always developed from primordia of more than one cell. Other modes of reproduction may be present.

For convenience bulbils may be grouped under three heads: those which form an intercalary primordium of several cells; those which typically originate from a primary spiral; and those that are produced by a perpendicular branch or branches which do not form a spiral.

As has already been pointed out the distinction between simple bulbils and compound spores on the one hand, and the more complex bulbils and sclerotia on the other, is not always definite, and in certain instances it is difficult to determine to which category a given structure belongs. *Compound spores* are reproductive bodies of more than one cell, having a more or less definite form, and are usually the result of a successive or simultaneous division of a single cell. On the other hand, *sclerotia* are compact bodies capable of reproducing the plant and formed rather by the massing together of vegetative filaments, forming a pseudoparenchymatous tissue, but not developed from a group of more or less definitely related cells. Moreover, the individual cells of a sclerotium are not at all spore-like or independent of each other. *Bulbils*, are reproductive bodies, more or less definite in form and mode of development, and normally derived from primordia of more than one cell, rather than the result of successive or simultaneous divisions of a single cell, and their individual cells are more or less independent and spore-like.

Papulospora immersa n. sp.

PLATE 10, FIGURES 17–25.

Mycelium white, septate, scanty, procumbent, growing in or on the substratum; bulbils, light brownish-yellow, irregular, 88–150 μ in diameter, but very variable, sometimes the long axis exceeding 260 μ, often immersed; central cells large 28–55 μ in diameter, angular, with conspicuous oil globules; 50–70 cells in surface view, but in irregular forms 100 cells, no differentiation of internal and external cells. No other mode of reproduction at present known.

On horse and dog dung from Cambridge, Massachusetts, and rabbit dung from Innerkip, Ontario.

Both the bulbils and the mycelium usually grow more or less below the surface of the substratum. The former are often found immersed more than a centimeter. It is easily distinguished from *P. polyspora* by its mode of development and from *P. pannosa* by its color, the latter being black. It resembles most nearly *P. irregularis*, from which it may be distinguished by its darker color, the size and conspicuous contents of the cells of the bulbils and the fact that the latter become more or less imbedded in the substratum.

The mycelium, since it is formed largely in the substratum, is inconspicuous in tube-cultures and is composed of large swollen hyaline cells, densely filled with oil globules and often much contorted (Figure 17, Plate 10). In older cultures the cells lose their contents.

This fungus was grown on different kinds of media, but could not be induced to develop any other mode of reproduction. It grows well on bran and horse dung agar, the bulbils often becoming very large and numerous just below the surface of the substratum, forming almost a continuous layer, and often producing a more or less hard crust. In contrasts of mycelia in plate cultures, a marked heaping of the hyphae occurs where the two mycelia come together, and the bulbils seem to be somewhat larger, and more irregular in this region, but no other marked difference was observed.

The bulbils.— The primordium of the bulbil consists of one or more intercalary cells which become much enlarged. For example, Figure 17, Plate 10, a later stage of which is seen in Figure 23, shows several such cells, all of which would have taken part in the formation of a somewhat elongated irregular bulbil, such as is shown in Figure 23. On the other hand, Figure 18 represents a primordium which consists of a single cell, and Figures 19–22 are further stages in its development. In the latter case a more or less spherical bulbil is the result (110–148 μ in diameter), while in the former it is more irregular, often exceeding 200 μ through the long axis. The method of enlargement, however, is exactly alike in both cases, that is, short lateral branches are produced from the bases of which are cut off a series of short cells which enlarge, becoming spherical at first and later, as the bulbil increases in size and the cells are subjected to lateral pressure forming a compact angular mass in the center. Occasionally the branches are replaced by cells which, arising as lateral buds, become spherical and in turn give rise to other buds, the lateral walls of which adhere closely and ultimately form a more or less

spherical or elongated bulbil with a fairly even margin, the central cells of which soon become angular. In either case all the cells are filled with conspicuous oil globules. At maturity there is no differentiation of central and cortical cells, but all are uniformly filled with food material, the central ones being larger, 28–35 μ in diameter, and more angular than those nearer the periphery.

Papulospora pannosa n. sp.

PLATE 6, FIGURES 20–25; PLATE 8, FIGURES 28–31; PLATE 9, FIGURES 18–20.

Mycelium white at first, becoming dark smoke-colored, 8–10 μ in diameter, somewhat shaggy; bulbils black, irregular, variable in size and outline, sometimes 350 μ in diameter, but usually considerably less; cells homogeneous throughout, 200–300 cells in surface view; primordium, a group of intercalary or terminal cells. No conidia observed.

On laboratory cultures of rabbit and goat dung, and on corn-cobs from Claremont, California.

Pure cultures of this fungus from about fifteen different sources were obtained and grown on various kinds of media and the mycelium from the different sources contrasted with each other, but thus far it has not developed any other mode of reproduction than the bulbils. This species is easily distinguished from most of the others by the color of its bulbils. The only other black form is that of *Cubonia bulbifera* from which it differs in size and the character of its outline, which is quite even and regular in the latter, as well by the fact that the hyphae are black at maturity.

The bulbils.— The mycelium which grows well on a variety of media in tube-cultures, appears somewhat shaggy, is white at first, gradually becoming dark smoke-colored, with prominent cross walls which remain rigid when the cells collapse (Figure 31, Plate 8). The hyphae which are 3–4 μ in diameter when young and hyaline, gradually increase in size until they are 8–10 μ in diameter, and have already become dark in color at the time the black bulbils are produced. During the formation of the latter, the hyphae become much distorted, and divide into a series of short, somewhat inflated cells which are separated by constriction at the septa (Figure 21, Plate 6), somewhat after the fashion of *Cubonia bulbifera*, but the successive cells of these series are much more irregular and of greater diameter. These enlarged cells send out lateral branches (Figure 18, Plate 9), from

which are cut off short basal cells which assume a spherical form, become swollen and may produce other branches similar to the primary ones. This mode of development is illustrated by Figures 20–24, Plate 6, and Figures 18–19, Plate 9. Instead of the enlarged cells producing branches, however, other cells may arise laterally from them by gemmation, become spherical, and may in turn give rise to others in a similar fashion. In either case the lateral walls of adjacent cells eventually adhere firmly, thus forming a compact group, each cell of which is almost spherical at first, but later becomes irregular. The further multiplication of the peripheral cells is subject to considerable variations. Not infrequently the primary or secondary branches, owing to local variation, grow much faster than others and thus produce more cells in that region of the bulbil. If there are several of these points of special activity, the mature bulbils may be quite irregular in outline. Occasionally a bulbil is formed from a single lateral branch (Figures 28–30, Plate 8), new cells being formed by a process of budding or by short branches as in the other cases. Ordinarily, at maturity, they are more or less spherical or somewhat elongated, their margins roughened by projecting cells (Figure 20, Plate 9) and are very variable in size, sometimes as large as 350 μ in diameter. There is no differentiation between the internal and external cells as far as contents are concerned. The central cells are, however, as a rule, larger and more angular.

Papulospora irregularis n. sp.

PLATE 9, FIGURES 11–17.

Mycelium white, more or less procumbent; bulbils hyaline, becoming light straw-color, somewhat spherical (140–170 μ in diam.) to irregular in outline (250–300 μ in diam.), margin very uneven; primordium a group of intercalary cells.

On rat dung, Kittery Point, Maine.

A pure culture of this species was comparatively easy to obtain. In the hyphae, which are hyaline, procumbent and inconspicuous, certain intercalary cells become enlarged and, by a process of budding, these give rise to other cells which in turn may produce still others. Sometimes short lateral branches are produced, the basal cells of which enlarge and take part in the formation of the bulbil (Figure 15, Plate 9). The young bulbils are colorless, covering the substratum, but in older cultures they turn light straw-color. They are usually somewhat spherical in form, measuring 140–170 μ in diameter, but

frequently run into irregular sclerotium-like bodies, 250–300 μ in diameter. In old cultures the hyphae often form a felted mass over the substratum. This mode of development is similar to that of *P. pannosa*, from which, however, it is easily distinguished by the color of the mycelium and bulbils, those of the latter species being black. It also resembles *P. immersa*, but it is lighter in color and does not have such large cells with conspicuous oil globules and the bulbils are not immersed in the substratum. Figures 11–17, Plate 9, illustrate the mode of development of this bulbil.

Papulospora spinulosa, n. sp.

PLATE 9, FIGURES 1–10.

Mycelium white, scanty, septate, procumbent, becoming slightly brownish when old, 3.5 μ in diameter, the old hyphae somewhat larger; bulbils hyaline until well developed, at maturity light chocolate-brown, somewhat spherical, 55–88 μ in diameter, 50–60 cells in surface view; primordium a coiled lateral branch which remains prominent throughout the development, becoming empty and showing slight thickenings in the walls. No other means of reproduction known.

On rat dung, Kittery Point, Maine.

This fungus was found on a gross culture of rat dung obtained from Kittery Point, Maine, and has been grown for about three years on various media without producing any reproductive body other than bulbils. The mycelium is white and grows quite sparingly on most media. It has been found that bran agar or rat dung agar is the best nutriment on which this species will grow.

The bulbils.— During their early stages of development the bulbils are hyaline until they are about half grown, at which time they begin to turn a light brown and at maturity assume a chocolate-brown color, often covering the whole substratum with several layers, so that all appearance of hyphae is lost sight of, except around the margin where a white zone about 5 mm. in width indicates the actively growing region of the mycelium and the formation of new bulbils. In the process of development a short lateral branch coils up, usually crosier fashion (Figures 1–4, Plate 9), although occasionally the tip somewhat overlaps, as shown in Figure 3, Plate 9. The primary loop varies greatly in size, as may be seen from a comparison of Figure 1 with the other figures representing the development, all of which are drawn on the same scale, but even these large open

primordia form eventually quite close coils. The helix which consists of one to one and one-half turns, divides into cells from which short lateral branches are produced, usually growing towards the center, rarely outward (Figures 5–7, Plate 9). These branches twine and intertwine, the lateral walls adhering firmly so that eventually a somewhat spherical body is formed which superficially resembles the sporangium of a fern. The cells of the original spiral are more prominent than the others, usually slightly elevated with well marked walls, and correspond to the annulus, as will be seen from Figures 9–10, Plate 9. Figure 10 is a view of an immature bulbil, looking down on the "annulus," while Figure 9 is a side view of the same. At maturity the bulbil, which is nearly spherical, is 55–88 μ in diameter. The cells of the primary coil usually become empty and lighter colored, showing slight thickenings scattered over their surface, occasionally projecting slightly, thus giving the appearance of minute spines.

Sometimes a lateral hypha divides dichotomously and each branch coils up and produces a bulbil. Similar branches may be produced directly from the superficial cells of a bulbil (Figure 8, Plate 9). The mode of development in this form resembles that of certain species of Urocystis, such as *U. cepulae*, the common onion smut, in which a lateral branch coils up, making about one turn, and this divides into cells from which secondary branches are given off. Figures 4, 5, 6 and even 7, Plate 9, might almost equally well illustrate the development of *Urocystis cepulae*.

Papulospora coprophila, nov. comb.

Helicosporangium coprophilum Zukal ('96).

Plate 10, Figures 1–16.

Mycelium white, septate, flocculent, abundant, persistent; bulbils, dark brown, more or less spherical, 30–40 μ (rarely 60 μ) in diameter, with one to four (sometimes as many as 10) large central cells surrounded by a cortex of empty colorless or slightly brownish ones; primordium spiral, of one to four turns, the end cell usually becoming a central cell. Conidia on bottle shaped sterigmata, frequently in white tufts scattered over the surface of the substratum.

On onions, straw, horse dung, etc., Cambridge, Massachusetts, and California.

Onions have proved very productive as a substratum for bulbils. Some onions obtained from the Boston market which had been shipped

from New York State, produced several different kinds and among them *P. coprophila* which has been secured from at least ten different sources, not only on onions, but frequently on horse dung and straw. It grows readily on potato and bran agar, but, like many of the other species, after continued artificial cultivation the mycelium becomes scanty and the bulbils few. In such cases it can be rejuvenated by growing on a gross culture of sterilized fresh horse dung, on which the mycelium is developed luxuriantly and becomes flocculent, producing bulbils and conidia abundantly.

This species appears to be the same as that described by Zukal ('86) under the name of *Helicosporangium coprophilum* which he found growing on horse dung. The general appearance of the bulbils of these two forms, their size, color, and at least one phase of their development seem to be identical. The form under consideration, however, differs from the description given by Zukal in producing a copious supply of flocculent hyphae. This may be due to the differences in the conditions of cultivation. *P. coprophila* resembles in mode of development the species referred by Eidam to *Helicosporangium parasiticum* Karsten, but the bulbils of the latter are brick-red, with yellowish cortical cells which, judging from the figures, are much less prominent than in the present form. The only other close allies are *P. parasitica* and *P. spinulosa*, the former easily distinguished by its single large central cell, the latter by its mode of development, and the presence of slight thickenings in the walls of the cortical cells.

This form develops sparingly on very moist substrata. On nutrient potato agar containing sugar, however, or on fresh horse dung, it grows well. Contrast cultures of mycelia from different sources yielded nothing more than additional variations in the filaments and bulbils. The former grew much more luxuriantly at the points of contact of the two sets of mycelia.

The bulbils.— A short lateral branch coils up, making about one or one and a half turns, the end cell enlarges, becomes spherical and frequently turns brownish. As it continues to increase in size its two lateral faces protrude more or less conspicuously and may even become subpendent, as in *P. parasitica* (Figure 4, Plate 5). These projections, however, often behave differently from those of the latter, since they are frequently cut off and thus form other enlarged central cells. Sometimes the second or even the third cell of the coil enlarges and takes part in the formation of the central cells. Those that do not enlarge grow out laterally over the surface of the central cell or cells and eventually completely enclose them. Figures 13–15,

Plate 10, show what appear to be arrested forms of this mode of development, all of which have brownish walls. These conditions resemble somewhat the mode of development figured by Zukal ('86).

About three or four days after inoculation on fresh nutrient agar which contains sugar, there frequently appears a spiral primordium of three or four turns, as shown in Figures 1–6, Plate 10, which divides into cells from which short secondary branches are produced, or other cells are formed by gemmation, so that eventually the spiral is enclosed by them. The cells of the spiral enlarge and usually lose their characteristic form. The lateral walls of the superficial cells adhere firmly together, so that eventually there comes to be one to four (sometimes as many as ten) large central cells, surrounded by a cortical layer of empty and often colorless cells (Figures 10–11, Plate 10). The development of the spiral may be checked at nearly any stage of its formation and thus certain variations in the form and number of the central cells of the bulbil may result. This variability in the formation of the spiral seems to be largely due to the character of the medium which, when favorable, usually produces quite regular primordia with the maximum number of coils, while under less favorable conditions, or after the substratum has been once run over with the hyphae, many variations are found. Some of the spirals are loosely coiled (Figures 1–2, Plate 10), while others are close and compact (Figures 4, 6, Plate 10). Although the primordium usually loses its spiral form early in its development, it is occasionally found surrounded by an irregular layer of cells, as shown in Figure 8, Plate 10. These bulbils resemble somewhat the primordium of a perithecium, like that of Melanospora as shown in Figures 5–6, Plate 3. On account of this resemblance an effort was made to induce them to develop into some perfect form, but although many and varied kinds of experimentation as to media, moisture and temperature, were tried, all efforts proved unsuccessful.

There are also associated with this bulbil spherical or slightly ovoid conidia, on bottle shaped sterigmata, identical with those found in connection with the melanosporous forms. These conidia, which frequently appear on conspicuous white tufts of hyphae scattered over the surface of the substratum, may be formed individually, in chains, or occasionally in a moist atmosphere may cohere at the ends of the sterigmata in a spherical mass. Although, as a rule, the sterigmata occur laterally on the walls of the hyphae, they are often found clustered on irregularly swollen branches and exhibit all the variations referred to below in connection with *P. aspergilliformis*,

although the characteristic "Aspergillus-like" fructification illustrated in connection with the latter has never been observed. These conidia were picked out with Barber's apparatus and transferred to nutrient tubes where they germinated and produced mycelium on which bulbils developed. In this respect they differed from those of *P. aspergilliformis*, which, although repeated efforts were made, could not be induced to germinate.

When these bulbils are crushed the contents of the large central cells escape, surrounded by a thick endosporium (Figure 11, Plate 10). These cells germinate readily in Van Tieghem cells (Figure 12, Plate 10).

Papulospora rubida n. sp.

PLATE 8, FIGURES 12–27.

Mycelium white, procumbent or slightly aerial on some media; bulbils more or less spherical, 30–40 μ in diameter, with 2–5 large central cells surrounded by a layer of empty cells which usually retain their yellowish red color, at maturity the whole culture has a brick-red aspect; primordium a spiral, with many modifications; conidia on bottle-shaped sterigmata, but not formed in white tufts.

On dog dung from Buenos Ayres.

This species was obtained from a pure culture received from Dr. Thaxter, which he has had growing for a number of years. It was originally found on dog dung from Buenos Ayres. In general it resembles *P. coprophila* in size, form, and mode of development. It is easily distinguished, however, by the appearance of the culture. The mycelium is more or less procumbent and the bulbils give the whole substratum a brick-red aspect, in old cultures forming a leathery incrustation which often cracks as the medium dries up. The mycelium of *P. coprophila*, on the other hand, is flocculent, filling the whole lower part of the test-tubes in slant cultures, and the bulbils give the culture a dark brown appearance. The cortical layer is colorless and more definitely marked in the latter species.

The hyphae of the form under consideration vary from 3–14 μ in diameter and, especially in old cultures, have well marked cross walls. Large swollen intercalary cells (Figure 24, Plate 8), are often formed, which seem to act as storage cells, as they are densely filled with granular, protoplasmic material and oil globules.

The bulbils.— A short lateral branch coils up spirally usually making one to one and a half turns (Figures 12–15, 21, 22, 27, 25a, Plate 8) and divides up into cells all of which become more or less swollen.

One or more of these cells, as a rule the first or second or both of them, increase in size beyond the rest, becoming densely filled with granular material and oil globules, while the other cells grow out laterally (Figure 16, Plate 8) and eventually enclose the enlarged cells in a manner similar to that of *P. coprophila* and *P. parasitica*. It sometimes happens that when the end cell enlarges, protuberances are produced from the lateral sides, which may even become subpendent, as in *P. parasitica* (Figure 26, Plate 8). The development of the cortical cells is shown in Figures 16, 21, 22 and 27, while Figure 25 is a median section and Figure 18 a surface view of the mature bulbil. Thus at maturity the bulbil is more or less spherical, 30–40 μ in diameter with 1–5 (usually 2 or 3) large central cells each of which varies from 10–14 μ in diameter (Figures 16, 25, Plate 8), surrounded by a cortex consisting of a single layer of empty cells, rarely more, which is often incomplete. The walls of the cells of this cortical layer usually retain their color.

Occasionally the short lateral branch instead of making but one or one and a half turns continues the spiral until from three to five turns are formed (Figures 17, 20, Plate 8). From the cells of the spiral are produced others laterally by budding, which eventually adhere to each other laterally, thus forming a wall about the spiral. This is similar to the process observed in connection with *P. coprophila*.

This species also produces conidia on bottle-shaped sterigmata similar to those described in *P. coprophila*, but they do not, as far as the writer has observed, occur in white tufts scattered over the substratum as they do in the last named species.

Papulospora sporotrichoides n. sp.

PLATE 12, FIGURES 1–41.

Mycelium white, procumbent, usually scanty; bulbils dark chocolate colored, somewhat spherical or flattened, 21–36 μ in diameter, primordium a spiral of one to two turns, with conspicuous oil globules, the spiral sometimes not well marked. Conidia and conidiophores of the Sporotrichum type.

On Live Oak chips (*Quercus agrifolia*) and corn cobs from Claremont, California, and Maple chips from Newton, Massachusetts.

The bulbils.— In the development of the bulbil a short lateral or terminal branch coils up, divides into a number of short cells with walls well distinguished, forming a close spiral of two or, rarely, three turns. This process is illustrated by Figures 1–9, Plate 12. During

the very early stages of development, the primordia are colorless, somewhat larger than the ordinary hyphal threads with more granular material. The walls, however, begin to turn brown shortly after division takes place. In Figure 5, for example, the walls are distinctly colored. In the mature bulbil the spiral form can sometimes be recognized (Figure 8, Plate 12), but more frequently, owing to the unequal enlargement of the cells composing the coils, or some modification in the development which will be spoken of later, all trace of it is lost.

The development of these bulbils was carefully followed in pure Van Tieghem cell cultures, and many interesting modifications were observed. Quite frequently, as illustrated in Figures 12–14, Plate 12, before the spiral has completed one turn or the walls of the individual cells thickened, one of the cells, usually the third or fourth from the tip, grows out into a vertical branch and coiling divides into cells similar to the first. The second coil may repeat this same process, so that two or three or even four coils like that which is shown in Figure 14, Plate 12, are formed one above the other, each producing a separate bulbil. These usually continue their development independently of each other, but not infrequently the primordia overlap and a single "compound" bulbil of two or three spirals, as the case may be, is the result. Occasionally this secondary branch is produced on the opposite side of the cell so that it grows into the concave portion of the first coil as shown in Figure 15, Plate 12. In some instances a single coil only may be formed, the cells of which enlarge as usual (Figures 19–25, Plate 12) becoming divided during the process, by thin cross partitions which are at first hardly visible without staining. The multicellular bulbil thus produced, does not become dark at once like the normal type but remains hyaline for some time, slowly changing color and only after it has become fully mature does it assume the dark brown tint of the more common type from which, however, it is eventually indistinguishable.

The Conidia.—A conidial form of reproduction, which usually appears on old cultures after a large number of bulbils have been produced, is also connected with this fungus. These conidia are of the Sporotrichum type and were obtained from pure cultures by the transfer of individual bulbils. It seemed desirable, however, to obtain the bulbil-type from germinating conidia in order to eliminate all chance of error; but this was found unexpectedly difficult for the reason that single spores isolated by Barber's apparatus refused to germinate although cultivated in varied media. The conidial form

is as a rule scantily developed in older cultures only, but by using a special nutrient composed of a decoction of bran, Spanish chestnuts, horse dung and rotten wood hardened with agar, an abundant production of conidia was obtained after two months, the conidiophores (Figures 35–36, Plate 12) rising well above the substratum at the margin of the culture, so that large quantities of spores were readily obtained in an absolutely pure condition. Cultures of these yielded about two per cent of germinations after twenty days.

The development of these germinating conidia (Figures 38–41, Plate 12) was continuously followed in Van Tieghem cells until bulbils were produced on the mycelium derived from them.

The conidiophores (Figures 35–36, Plate 12) which are colorless at first but become light grayish brown at maturity, are larger (3.5–4 μ in diameter) than the other hyphae from which they arise, with quite irregular walls producing numerous lateral conidia which rest either upon short stalks or upon little projections of the wall of the conidiophore, or are completely sessile. The conidia, which are also colorless at first, but become the same color as the conidiophore, are ovoid, 4×7 μ, with smooth, fairly thick walls. During germination, they swell so as to be almost spherical in shape (Figures 39–41, Plate 12).

Papulospora cinerea n. sp.

PLATE 8, FIGURES 1–11.

Mycelium white, septate, procumbent, forming a felted mass over the substratum; bulbils steel-gray or slate-colored, somewhat spherical and flattened, 21–36 μ in diameter, with three or four large angular central cells, and a layer of fairly regular cells forming a cortex, but of the same color as the others; the primordium a spiral of one or two coils. No conidia known.

On gross culture in the laboratory, Cambridge, Mass.

This fungus was found running over a gross culture in the Cryptogamic Laboratories at Harvard University by Dr. Thaxter and has been kept growing as a pure culture for more than ten years. It is easily distinguished from any of the others by the steel gray or slate-color of the bulbils, which are round, somewhat flattened in form, and measure 21–36 μ in diameter, in which respects they resemble those of *Papulospora sporotrichoides*. The mycelium is white, procumbent, forming a felted mass over the substratum, the slate-colored bulbils being scattered among the white hyphal filaments, finally giving the whole culture a bluish gray or steel-gray appearance. When young

the hyphae are closely packed with oil globules which escape into the water when the filament is ruptured, and might be mistaken for spores.

The bulbils.—A short lateral branch coils up, usually making one or two turns, rarely more, and frequently less than two, and divides into a number of short cells from which secondary branches are produced, or from which individual cells are formed by budding (Figures 7–8, Plate 8). In either case, spherical cells which gradually increase in size, are developed, and the lateral walls adhere closely to each other. The original coil, the cells of which in the meantime have become much enlarged and filled with granular material and oil globules, is thus eventually completely surrounded. At maturity three or four large central cells may be distinguished which have become angular by pressure, surrounded by a layer of fairly regular cells which are also usually somewhat angular except the outer walls. It often happens that when one turn is made by the primordial coil, the secondary branches begin to form, while at other times two or more turns are formed before this happens. Between these two extremes a number of variations are found. Not infrequently the lateral branch becomes divided into four to eight cells and may or may not be coiled at the end, and from these, secondary branches are produced which coil around each other and around the original branch, dividing and subdividing, the lateral edges eventually adhering closely, and producing a more or less elongated bulbil (Figures 4–6, Plate 8). This process also inhibits the further growth of the coil. An extreme instance of this is shown in Figure 6, Plate 8, where several cells are seen to take part in the formation of lateral branches. Bulbils formed from a primordium of this type are elongated, irregular, and larger than those formed in the usual way.

Although this species was grown on a great variety of nutrient media, it could not be induced to develop any perfect form or even another imperfect type.

Papulospora parasitica nov. comb.

Syn.: *Helicosporangium parasiticum* Karsten. (nec Eidam.)

PLATE 5, FIGURES 1–17.

Mycelium septate, white, flocculent; bulbils light brown, nearly spherical, 15–21 μ in diameter, with a single large central cell surrounded by a single layer of empty colorless cells; primordium a spiral, coiled crosier-fashion.

On bread, Cambridge, Massachusetts; mouse dung, Duarte, California.

This form which appears to be identical with *Helicosporangium parasiticum* Karst. was found by Dr. Thaxter on bread in Cambridge, Massachusetts, and kept as an herbarium specimen, but was too old to be resuscitated. The writer also found it on a gross culture of mouse dung in an old paper bag obtained from Duarte, California. This culture was so overgrown with Penicillium and other foreign material which grew so much more rapidly than the bulbiferous fungus that it was difficult to get it pure. This was finally accomplished by using a gross culture of sterilized peas on which the mycelium of the bulbil grows quite rapidly.

The bulbils.— The development of the bulbils, which are produced in large numbers, agrees in all essential points with the original description and figures of Karsten ('65). Short lateral branches of the hyphae coil up crosier-fashion and, although quite open at first, soon close up, forming a close coil which divides into short cells, all of which increase in size to a certain degree. One of these, usually the end cell, but not infrequently the second, enlarges more rapidly than the others and becomes a "central cell," the remaining members of the coil forming a ring or "annulus" around it and becoming firmly attached to the side of the original lateral branch. As this central cell increases in size more rapidly than those of the coil, considerable lateral pressure is exerted and consequently protuberances usually appear on each side of it which usually becomes subpendent and subsequently may divide into two or three lobes (Figures 4, 5, 9, 10, Plate 5). As this tension is released, probably through the increase in size of the "annulus," the large central cell loses its lobed appearance and assumes a spherical form (Figure 11, Plate 5) and may later become somewhat angular.

In the meantime the cells composing the "annulus" begin to grow out laterally, extending over the surface of the large central cell, and in the mature bulbil completely corticating it, the walls adjacent adhering laterally. Sometimes there is a small pore left at one or both of the centers of the lateral faces of the central cell and through them at germination the germ tube grows, but this is the exception and is probably one of the incomplete stages of development that will be spoken of later.

During the early stages of development and even until they have almost reached their full development these bulbils are colorless, but eventually they become light brown. At maturity they are nearly

spherical in form, consisting usually of a single large central cell about 10–14 μ in diameter, densely filled with granular material and oil globules, and surrounded by a single layer of empty colorless cells, the whole bulbil measuring 15–21 μ in diameter. Although the foregoing description of the mode of development of the bulbil is the characteristic one, the process may vary considerably in different cases. Occasionally there appears a tendency to form a helix, at other times a protuberance from the central cell develops only on one side or not at all, and quite frequently the "annulus" is incomplete, or the cortical cells that are derived from it fail to cover the whole central cell. It would thus appear that the development of the bulbil may be arrested at nearly any stage, and these arrested forms, under proper conditions, will germinate almost immediately.

In Van Tieghem cells these bulbils germinate in 24–36 hours and send out one or two germ tubes, as shown in Figures 15–16, Plate 5, which arise from the central cell only. The germ tubes usually proceed from that region where the marginal cells meet or, as sometimes happens fail to meet, leaving two small pores, as already mentioned. In incompletely developed bulbils, the germ tube seems to come out from any point offering the least resistance.

Conidia-like bodies were occasionally found connected with this fungus when grown on straw. A short lateral branch, which not infrequently becomes septate (Figure 17b, Plate 5), enlarges at the end and from it an ovoid cell (4.5 × 6.5 μ) is abjointed. Unfortunately these were produced so rarely that their germination and further development could not be observed. Figure 17, Plate 5, however, shows a direct connection between these "conidia" and a bulbil.

This form agrees in all respects with the original description and figures of *Helicosporangium parasiticum* (Karsten '65) except that it is saprophytic and that no "endospores" are found in the central cell. As already stated, Karsten was of the opinion that the contents of the cortical cells passed into the central cell, either directly or by diffusion and as a result of the union of these different protoplasmic bodies the spores were formed. If the account given by Karsten is correct, in all its details he was not dealing with a bulbiferous form at all. It would seem, however, that later writers are probably correct in considering them as such, since Karsten may have been misled by the presence of more or less regular oil globules, such as occur in this and other species and which might easily have been mistaken for endospores. On the other hand, it is by no means impossible that he was

dealing with a form related to Monascus, which has not been recognized by subsequent investigators. Since, however, the morphology and development of his "Helicosporangium" corresponds so exactly with that of the bulbil under consideration and since also the "parasitism" of his plant on "beets," seems at least very questionable, the writer feels little hesitation in concluding that he was dealing with a bulbil, in all probability identical with the one under consideration.

Harz ('90), in his account of *Physomyces heterosporus* (*Monascus heterosporus* (Harz) Schröter), is of the opinion that this plant is closely related to *Helicosporangium parasiticum* Karsten, and further suggests that *Papulospora sepedonioides* Preuss, belongs near this fungus also, the difference consisting in the fact that the central cell of the latter is said to contain but one or only a few "endospores."

The bulbils described and figured by Zukal ('86), under the name of *Dendryphium bulbiferum*, also resemble this form in appearance and mode of development, except that it does not produce the lateral protuberances from the developing central cell, at least they are not mentioned or figured, and that it is described and illustrated as being intimately connected with hyphae producing spores of the genus Dendryphium.

In this connection it may also be mentioned that the spores of *Stephanoma strigosum* Wallr. (*Asterophora pezizae* Corda, *Synthetospora electa* Morgan, *Asterothecium strigosum* Wallr.) show stages that resemble quite closely certain conditions in the development of *P. parasitica*. Figure 35, Plate 5, for example, is an abnormal spore of Stephanoma and, except for its size and color, might easily be taken for an imperfectly developed bulbil of the form under consideration, such as is represented by Figure 14, Plate 5.

A corresponding resemblance may also be seen between imperfectly developed bulbils of the present species, in which the cortical cells have failed to surround the central cell completely, and the immature bulbils of *Aerospeira mirabilis* described above.

Papulospora aspergilliformis Eidam.

Plate 7, Figures 1–20.

This bulbil was obtained from several different sources, chiefly on onion leaves, wheat chaff, and oat straw from the vicinity of Cambridge, also on straw from Claremont, California. It is not at all rare and can easily be obtained by placing straw in a moist chamber. It is readily distinguished by its relatively large, irregular, sclerotium-like

bulbils. Pure cultures from a half-dozen different sources were made by the methods already described, and kept under cultivation on a variety of media.

The septate mycelium grows very slowly on nearly all substrata, producing the best results on bran agar, and on sterilized fresh horse dung on which it becomes somewhat flocculent. The primary mycelium grows on the top of the substratum, or just below the surface, and sends up lateral branches into the air. It is these lateral branches that produce its peculiar Aspergillus-like fructification. The primary mycelium becomes very large, usually somewhat contorted and packed full of granular material and oil globules. The hyphae, which anastomose readily often forming a sort of network, measure as much as 11 μ in diameter, and some of the swollen lateral branches 17 μ (Figure 4, Plate 7). Occasionally, especially in the young hyphae, there occur large swollen intercalary cells containing oil globules and other food material (Figures 17–18, Plate 7). These seem to be cells for the storage of food.

The bulbils.— The mycelium grows out evenly in all directions from the point of inoculation. In about two or three weeks (on horse dung, in about a week), small brownish-red spots appear near the margin of the mycelial growth. These are young bulbils, and on closer examination they are found to develop as follows. A short lateral branch (Figures 2–3, Plate 7) well filled with nutrient material, sends out branches which twine about each other. The former sometimes coils at the tip but this seems to be incidental. These secondary branches may come off near the base of the lateral branch (Figure 3, Plate 7), and by twining about the primary hypha may incorporate it into the bulbil. More often, however, the secondary branches come off a short distance from the hypha (Figures 2, 4, 6, Plate 7), so that, especially in the early stages, it is evident that they are on short pedicels. The secondary branches intertwine with each other, and divide up into short cells, their lateral walls adhering firmly to those of their neighbors and eventually forming a compact mass of uniform cells. At maturity these bodies superficially resemble true sclerotia perhaps more nearly than they do typical bulbils, but they are developed from a group of cells composing the primordia, and not from a mass of interwoven hyphae from different sources. They vary considerably in size and shape, some of them being nearly spherical, about 100 μ in diameter; but most of them are irregular in form, reaching in old cultures 570 $\times$ 750 μ. There is no differentiation between the marginal cells and the central cells. Microtome

sections show that the bulbil is uniform throughout (Figure 20, Plate 7) all the cells containing protoplasm, and under favorable conditions capable of sending out germ tubes. In this respect it differs from the typical sclerotium, which usually has a compact layer of several cells in thickness (the rind) which forms the margin. The primordia are colorless at first (Figures 2–4, Plate 7), then light-yellow, later ruby-red, and finally reddish brown and opaque.

In this as in most other bulbils the process of development may vary greatly. Figure 1, Plate 7, shows the primordia of three bulbils, two of which and possibly the third also, would probably have grown together, forming a large, irregular, sclerotium-like body. This phenomenon occurs quite frequently, giving rise to a variety of forms, which vary with the number of the initial primordia taking part in their development, their proximity, and the inequality of their development. In such cases each primordium develops independently, until its lateral branches intertwine with those of one or more that lie adjacent to it, a compound bulbil finally resulting, in which the several origins are indistinguishable.

Aspergillus-like fructification. Conidia are frequently produced both on Aspergillus-like heads and also laterally, on the sides of the hyphae (Figures 10–11, Plate 7). The latter are usually isolated, sometimes irregularly grouped. The conidiophores arise from erect lateral branches, and are frequently septate; rarely branched. They are very minute, so that one can detect them only with difficulty, even with a good hand lens. The length of the conidiophore varies greatly, some being quite short, others so long that it is difficult to trace them to their origin. The swollen head of the conidiophore is usually spherical, or nearly so, and on it are arranged somewhat irregularly numerous simple sterigmata. These vary slightly in size and shape, but always have a broad base and taper more or less gradually, often to a point, at the distal end. The relative length of the vertical and transverse diameters of the swollen base varies somewhat, so that one may find gradations in shape from almost spherical to napiform. The conidia are nearly spherical, sometimes ovoid, smooth, colorless, minute, occurring in chains, and dropping off very readily; but in moist atmosphere the conidia, instead of being produced in a chain, frequently adhere and form clusters much like those of Hyalopus.

There are many variations in the arrangement of these conidia, which may, for example, arise, as is shown in Figure 9, Plate 7, terminally and laterally on irregularly clavate extremities of hyphae.

Occasionally a conidiophore may form an intercalary swelling with conidia on it, as if it were a secondary head (Figure 10, Plate 7).

Chlamydospore-like bodies occur quite frequently. They are mostly intercalary but sometimes terminal (Figures 13-16, Plate 7). When young they are colorless, or opalescent, slightly swollen, ovoid cells, filled with granular material. At maturity they are usually more spherical and have thick brown walls (Figures 13, 15, Plate 7). Occasionally more than one cell takes part in the formation of these spore-like bodies. Figure 16, Plate 7, shows two such cells and Figure 5, Plate 7, a large number of "chlamydospores" closely packed together.

There are several forms that have Aspergillus-like fructifications, similar to those just described and which may be considered briefly at this point. As has already been noted, Eidam ('83) describes these structures in his account of *Papulospora aspergilliformis*, and also chlamydospores resembling those of *Acremonella atra* Sacc. (*Acremonium atrum* Corda.) such as are produced by *Melanospora cervicula*. Eidam, however, described two types of bulbils in *P. aspergilliformis*, a small one that develops in a manner similar to the form examined by the writer, and a large one, the primordium of which is spiral, resembling that described by Bainier ('07). It is quite possible that Eidam has here confused the primordia of two species the larger of which corresponds in all essentials to that studied by the writer. On the other hand his smaller bulbil would correspond more closely with that studied by Bainier.

Bainier ('07), in his article on *Papulospora aspergilliformis* also refers to its "Aspergillus-like" conidial fructification. According to his account the primordium of the bulbil consists of a short lateral branch which coils up spirally and eventually produces a more or less spherical bulbil. Under certain conditions of nutrition and moisture, however, the latter are said to produce large sclerotium-like bodies, which in turn may be induced to develop further and form perithecia, which are referred to the genus Ceratostoma. This form described by Bainier seems to be different from the one under consideration, since the bulbils of the latter do not develop by means of a spiral and are large and sclerotium-like. The present form, moreover, has been grown for nearly three years and during that time it has never been observed to produce any other type of bulbil than the one described. It has, however, produced in abundance conidia on Aspergillus-like conidiophores which sometimes occur in direct connection with the bulbil (Figure 8, Plate 7). This species has been compared

with material received from Professor Bainier by Dr. Thaxter, and the two forms have been grown on many and varied kinds of nutrient material for nearly three years during which time, as already mentioned, the American material has never been observed to produce small spherical bulbils; nor has the form received from Bainier developed the large sclerotium-like bodies which he describes, although every effort has been made to obtain them.

There is also a marked difference in the method of growth in these two forms. The mycelium of the American form grows very slowly on bran or corn agar, but fairly rapidly on horse dung, while Bainier's species grows rapidly on a variety of media. There is also a marked difference in the general appearance of the two while growing in cultures; the mycelium of the former being quite inconspicuous at first and often two or three weeks elapse before bulbils are produced. The two forms thus appear to be very probably distinct and there seems little doubt but that Bainier was mistaken in referring his species to *P. aspergilliformis*. Neither of these forms has associated with it Acremoniella-like Chlamydospores, such as Eidam describes and it seems not improbable that Bainier is right in believing that these spores do not belong to *P. aspergilliformis*, but are those of "*Acremonium atrum*" which although frequently associated with it are not a part of its life cycle.

The writer has under cultivation about a dozen pure cultures of *Acremoniella atra* obtained from different sources, some of which were closely associated with bulbils, and these have been grown for nearly three years under varying conditions of temperature, moisture, and nutrient material, the different mycelia having been contrasted on plate-cultures under various conditions. In no instance, however, have bulbils or Aspergillus-like conidiophores been produced.

Harz ('71) has described a form under the name of *Monosporium acremonioides* that produces chlamydospores and conidiophores similar to those of *P. aspergilliformis* Eidam, but not associated with bulbils, and states that the conidia were produced on secondary heads either sessile or short-stalked, like those of *Melanospora cervicula*. This latter character has been used by Costantin ('88) as the basis of a new genus, Harzia, into which he puts the foregoing species under the name of *Harzia acremonioides*. Later, in referring to *Papulospora aspergilliformis* Harz ('90) calls attention to the striking resemblance between the two spore-forms of this fungus and those of *Monosporium acremonioides* Harz, and suggests that, if they are the same, the name should at least be *Papulospora acremonioides*, although

he takes exception to the generic name on the ground, as will be seen later, that it does not correspond with the description of the genus by Preuss.

Lindau ('07) apparently is of the opinion that these two forms are the same and he creates a new genus, Eidamia, for their reception under the name *E. acremonioides* (Harz).

The conidial form of *Melanospora cervicula* resembles quite closely *Harzia acremonioides* in having its conidia on secondary heads and in producing Acremoniella-like chlamydospores, but differs in possessing bulbils and melanosporous perithecia. It is quite possible, however, that the two are identical. It is possible also that the so-called "Harzia type" of fructification, as seen in *M. cervicula* and the "Aspergillus-like" type as seen in *P. aspergilliformis*, are modifications of one and the same mode of reproduction: since on several occasions the writer has found in connection with the conidial fructification of *M. cervicula* instances in which secondary heads seemed to be lacking, but, owing to the fact that there was only a limited amount of material available, this point could not be absolutely determined. The perithecium of this form, however, is clearly of the melanosporous type, and can hardly be the same as the Ceratostoma described by Bainier.

The writer has under cultivation the *Mycogone ulmaniae* Potebnia, ('07) (*Chlamydomyces diffusus* Bainier) obtained by Dr. Thaxter from Liberia and kept in cultivation for over fifteen years. In addition to its large two-celled, warty, chlamydospores, this species also produces conidia on "Aspergillus-like" conidiophores similar to those of *P. aspergilliformis*.

Conidial forms similar to those above mentioned are also described by Möller ('93) in connection with the garden fungi of certain species of ants in the tropics.

Again, large chlamydospores, somewhat similar to those of *Melanospora cervicula* except that they are divided into two unequal cells, have been described by Berlese ('92) in connection with *Sphaeroderma bulbilliferum*. They differ from those of *Mycogone ulmaniae*, however, in being smooth.

Papulospora polyspora, n. sp.

PLATE 11, FIGURES 1–13.

Hyphae septate, hyaline, scanty, procumbent, 5–7 μ in diameter (sometimes as much as 9 μ); bulbils dark red-brown usually with a

thin mucilaginous film about each, eventually becoming a dry powdery mass, completely concealing the mycelium, more or less spherical, 119–122 μ in diameter, composed of closely compact angular cells, 150–200 cells visible in a surface view; cells homogeneous throughout. Individual cells of the bulbil eventually forming spherical spores, 17–22 μ in diameter loosely held together. No other spore-form known.

On straw, old paper, from California and cotton flowers from Cuba.

This fungus has been obtained from at least three different sources. It was found by Dr. Thaxter running over a gross culture of the flowers of Cuban cotton and also by the writer on gross cultures of barley straw from Claremont, California, and on old paper from Duarte, California.

The usual methods of obtaining a pure culture were employed here, after which the fungus was grown on various kinds of nutrient material, but it could not be made to produce any perfect form. Mycelia from widely different sources were contrasted in Petri dishes but no results were obtained except the production of certain abnormal enlargements and contortions of the hyphae, such as may frequently be observed in contrasting forms of even widely different species.

The mycelium of this fungus is white, inconspicuous, procumbent, the hyphae densely filled with coarse granules or oil globules. At a short distance from the margin of growth small white pustules are seen, which gradually become larger and more frequent as they approach the point of inoculation. These soon turn tan-colored, and are frequently associated with small drops of liquid of nearly the same color, which may often be seen surrounding a bulbil. At maturity these bulbils are almost spherical, 119–122 μ in diameter, composed of closely compacted angular, often irregular cells, uniform throughout, there being no distinction of a definite cortex. They occur in large numbers heaped together, covering the whole substratum and obliterating completely the naturally scanty mycelium. In older cultures they become a dry powdery mass.

The bulbils.— The formation of this bulbil is different from that of any of the others thus far considered, since they result not from the development of a single primordium but from the combined activities of several primary branches. One or more procumbent hyphae send up vertical branches which twine about each other (Figures 1–4, Plate **11**). Usually several of these branches arise simultaneously at a given point (Figure 3, Plate **11**) and as the bulbil increases in size, more and more of these take part in its formation, their extremities combining to produce the bulbil proper, while just above the substratum there may form a sterile supporting base, often with a

diameter nearly equal to that of the bulbil itself and composed of interlacing hyphal strands, which are partly made up of branches from the procumbent hyphae and partly by the branching of the original vertical ones. These supports or "stalk-like" structures vary in length, some being quite long (100 μ), while at other times the bulbils appear to be almost sessile on the horizontal branches. The primordia that are produced later, are hindered in their upward growth by the presence of the first formed bulbils, which, however, are soon broken away from their attachments and pushed up so that eventually several irregular layers of independent spherical bodies are produced, the oldest ones being on the surface. Whether the vertical hyphae first formed fuse at the apex could not be determined. They evidently receive some stimulus, for they begin to send out short branches in different directions, which in turn divide and subdivide, and these intertwine among themselves and, with other hyphae that grow up from the original horizontal branches, form an interlacing weft which becomes more and more compact, producing a hyaline, spherical body in which the walls are very thin and almost indistinguishable except after staining. As they increase in size they assume a brownish tint and finally a rich tan-color, during which time the walls gradually become more definite and eventually are well marked.

Since liquid media appeared to have a peculiar affect on the development of these bulbils, cultures were tried in large flasks on pieces of wood partly immersed in bran decoction, so that the effect of different degrees of moisture might be observed, as the mycelium spread from the liquid medium toward the dryer portions of the wood. Under these conditions it was found that the bulbils formed on the wood about three or four inches above the liquid, began to assume a paler aspect and soon became light straw-colored, instead of the dark tan of the normal bulbil. On examination it was found that the cells composing these pale bulbils, instead of being compact with angular walls as in the normal form, had rounded up and become spherical (17–22 μ in diameter), adhering very loosely by means of a mucilaginous material that had evidently been secreted by them, so that a very slight pressure would separate them into individual spores (Figure 8, Plate 11). The germination of these "spore-masses" was followed carefully in Van Tieghem cells — some crushed, others not — and it was found that nearly all the spores germinated in twenty-four hours, some producing one, others two germ tubes, which were hyaline and septate, becoming much branched (Figures 9–10, Plate 11). When allowed to remain adherent, the spore-mass sent out germ tubes in all

directions which shortly forced the individual spores apart. The bulbils were also germinated in Van Tieghem cells, but their germination was much slower and they produced comparatively few germ tubes which seemed to be chiefly from the superficial cells.

In water cultures the hyphae are usually larger and more densely filled with granular material, with numerous large swollen intercalary or terminal cells (Figures 9b–13, Plate 11). These cells are grouped together irregularly as if attempts were being made to form bulbils but they do not become compact. They often grow very large, as may be seen by a comparison of Figures 9b–13, Plate 11, all of which have the same magnification.

This development and final fate of the bulbil of *P. polyspora*, suggest a similar condition that is found in Aegerita. In *Aegerita Webberi* Fawcett ('10) the "sporodochia" which measure 60–90 μ in diameter, consist of an "aggregation of conidia-like, inflated, spherical, cells, 12–18 μ in diameter," resembling the conditions described for *P. polyspora*. The development of the latter on the other hand recalls also that of the sporodochium of *A. candida* Persoon (*Peniophora candida* Persoon) as described and figured by Lyman ('07) and it is possible that the two structures may be similar in nature.

OTHER RECORDED BULBIFEROUS FORMS.

In addition to those above enumerated several other bulbils or bulbiferous forms have been recorded, some of which have already been referred to, but which may here be again mentioned.

Papulospora Dahliae Costantin ('88). This species was found by Costantin on roots of Dahlia. Its bulbils appear to be somewhat like those of *P. coprophila*, brownish-red in color, with two or three large central cells surrounded by a layer of empty cortical cells. Conidia belonging to the genus Dactylaria are, however, said to be associated with these bulbils, although it is not evident that the species was cultivated in a pure condition.

Dendryphium bulbiferum Zukal ('86) has been mentioned on page 233, and also in connection with *P. parasitica*. The bulbils described and figured by Zukal are said to be directly associated with the conidia of a Dendryphium; but here, as in other forms studied by this author, there is no evidence that pure culture methods were used in studying the fungus.

"*Haplotrichum roseum* Lk." is also stated by the same author ('86) to be associated with bulbils said to be very similar to those of the

Dendryphium just mentioned; but here again pure cultures do not appear to have been used. As far as the writer is aware, moreover, this common hyphomycete has never been seen to be thus associated by any other observer.

Papulospora (Stemphylium) **Magnusianum** (Sacc.), (Michelia, I, 132) a form collected by Magnus in the Tyrol, distributed in Vestergren, Micr. Sel., No. 1150, and also figured by Saccardo in Fungi Italici, No. 934, should be mentioned in the present connection, since it is a typical bulbil and by no means a compound spore like that of species of Stemphylium.

Clathrosphaera spirifera Zalewski ('88), is a form which the author, although his observations are concealed in Polish text, appears to regard as bulbiferous, or as producing bodies comparable to bulbils, which are also associated with a species of Helicoon.

The writer has himself observed various other more or less ill defined types of bulbils, which have not been above enumerated, since they do not appear to be sufficiently well marked to warrant a definite name. "No. 170" for example (Figures 24–31, Plate 5), was found in California on straw from Claremont, and on old paper from Duarte. The fungus is characterized by an abundant white mycelium, the hyphae of which produce bulbil-like bodies consisting of a few cells each, as indicated in the figures. Their characters and development, however, are not constant and their exact nature is somewhat doubtful.

COMPOUND SPORES AND OTHER REPRODUCTIVE STRUCTURES WHICH RESEMBLE BULBILS.

Reference has already been made to the close resemblance which exists between the so called "spore-balls" of some of the Ustilaginales, and the structures under consideration; in fact it would be quite impossible to differentiate the spore-balls of Urocystis or Tuberculina from bulbils, as far as concerns their gross structure and method of development which may be exactly similar. They are, however, clearly distinguished in other ways; since in bulbils, spore formation is never preceded by any nuclear fusion, so far as is known; and furthermore the germination of bulbils in no way resembles that of the smuts; and there is never any indication of the formation of anything corresponding to a promycelium.

Attention has also been called to the fact that the compound spores

which are associated with the imperfect forms of many of the higher fungi, may bear a close resemblance to bulbils. Although compound spores may in general be distinguished by the fact that they normally arise as the result of the septation of a single cell, while in the production of bulbils two or more cells are primarily involved, to which others are added by a process of budding which may also be combined with secondary septation, it is not always possible to separate them with certainty. Spores like those of Stephanoma, referred to elsewhere, in which the empty superficial cells arise by budding, serve, however, to break down this distinction.

On the other hand, the more complicated types of bulbils are easily comparable to the simpler types of sclerotia, such as occur for example in *Penicillium Italicum*, *Verticillium agaricinum* and similar forms. Such sclerotia, however, result from the irregular and indefinite massing together of vegetative filaments, the densely compacted cells of which do not partake of the nature of spores, while the functional cells of bulbils are usually spore-like and act independently of one another at the period of germination.

Among the compound spores formed in connection with the imperfect conditions of higher fungi, several may be mentioned which have bulbil-like characteristics.

Stephanoma strigosum Wallr. a parasite on *Peziza hemispherica* which, as Dr. Thaxter informs the writer, occurs also on *Geaea hispidula* in this country and is connected with an undescribed hypocreaceous perithecial form, might very well be regarded as a bulbil of a simple type, since not only are its spores similar in their development, but, when mature, are hardly distinguishable from the more simple bulbils which are often produced, for example, by *Papulospora parasitica*.

Stemphylium macrosporoideum Sacc., which has been examined from cultures kept in the Cryptogamic Laboratories, produces a compound spore consisting of one large functional cell to which, at maturity, two or more empty ones are attached. In this condition it resembles very closely the bulbil of *Acrospeira mirabilis;* but in view of the fact that it develops as a result of the successive divisions of a single terminal cell, it must be regarded as a compound spore. Certain other forms also of Stemphylium as well as of Mystrosporium might well be mistaken for bulbils.

Hyalodema Eransii P. Magn., which von Höhnel has referred to *Coniodyctium Chevalieri* H. & Pat., produces a hymenium-like layer bearing compound spores which, except in color, are very like the

bulbils of *Papulospora sporotrichoides*. Their development, however, is clearly that of compound spores and not of bulbils.

Elaomyces oleï Kirchner ('88) a fungus found growing in poppy oil, produces a compound spore which consists at maturity of a large thick-walled functional cell, surrounded by several empty coherent cells, the whole resembling the bulbil of Acrospeira. If, as suggested by Kirchner, this body results from the coherence of several adjacent cells, it might well be regarded as a bulbil and not a compound spore.

Various other spore-forms might be mentioned which bear more or less resemblance to bulbils, but those above enumerated are sufficient for purposes of illustration. Before leaving bulbil-like forms, however, two or three additional types may be mentioned, the nature of which is not altogether clear, since they are neither compound spores nor typical sclerotia.

Aegerita Webberi Fawcett ('10), a fungus attacking scales on Citrus, produces, under certain conditions, bulbil-like bodies which consist of loosely coherent spore-masses closely comparable to those of the aberrant *Papulospora polyspora*, the development of which, under moist conditions, has been described above.

Sorosporella Agrotidis Sorokin ('88, '89), which attacks the larvae of Agrotis, fills the latter with loosely but definitely coherent cell-groups which might also be compared to those of *P. polyspora*.

Lastly, among structures which bear a striking resemblance to bulbils, the peculiar spore-balls of *Spongospora subterranea* (Wallr.) Johnson should be mentioned; which, although they might readily be taken for a species of Papulospora, have been shown to belong to the life-cycle of one of the Mycetozoa.

THE MORPHOLOGICAL SIGNIFICANCE OF BULBILS.

Opinions concerning the morphological significance of bulbils differ widely. Preuss ('51), Eidam ('83), DeBary ('86), Mattirolo ('86) all regarded them as normal structures which function as auxiliary methods of reproduction; while Karsten ('65), Zukal ('86), Morini ('88), and Baineir ('07) looked upon them as immature ascogenous fructifications of either perithecial or apothecial forms, believing that their arrested growth was due to unfavorable environment, and that, with proper nutriment, they might be able to complete their development.

Although it is possible that the last mentioned view may be correct in some instances, it is quite certain that in many cases, where both

bulbils and ascocarps are present, this cannot be the case, since the primordia and development of the two are widely different. Thus in *Cubonia bulbifera*, for example, the bulbil is produced from a group of intercalary cells, while the primordium of the apothecium is a spiral. In like manner *Melanospora anomala* develops bulbils which arise from intercalary cells, somewhat as in Cubonia, while the perithecia arise from free spirals.

It is quite possible, however, that in other cases, as for example in *M. papillata*, where the primordium of the bulbil and that of the perithecium are similar, they may be homologous. But even in such cases, the two primordia are distinguishable so early in their development, that it is more than probable that here, also, they cannot be regarded as immature ascocarps. Various attempts have been made by the writer to induce the bulbils of various species to continue their development and produce ascocarps. Many bulbils of *M. papillata* for example, that had grown larger than the more normal types, were isolated and placed on different media where they were exposed to different degrees of moisture, with this end in view. Similar attempts were also made with the bulbils of *P. coprophila*, in which the spiral bulbil-primordium might be supposed to suggest its ascogonial nature. In no instance, however, was any evidence obtained that would seem to point to the conclusion that they were to be regarded as anything but independent non-sexual propagative bodies, except that, in some instances they increased in size, sometimes becoming approximately half as large as perithecia. This enlargement, however, was unassociated with any structural differentiation such as always characterizes the developing perithecium.

Although Bainier reports that he was successful in inducing the bulbils of *Papulospora aspergilliformis* to develop directly into perithecia which he refers to Ceratostoma, the writer has been as unsuccessful with this species as with others, even when using material derived from a living culture received from Bainier by Dr. Thaxter.

In view of the careful and long continued experiments made by the writer in this connection, and his entire failure to obtain positive results, the assumption seems justified that ordinarily, at least, bulbils are not to be regarded as abortive ascocarps, but rather as an auxiliary method of reproduction that has been interpolated in the life history of certain fungi without definite relation to other forms of reproduction which they may possess; or if they have in reality been derived from some other reproductive body, that this was more probably some type of compound non-sexual spore, rather than the primordium of an ascocarp.

DISTRIBUTION AND OCCURRENCE OF BULBILS.

It is evident from the foregoing account that bulbiferous types are not only widely distributed, but are very readily obtained if sought for, and, like so many other types among the Fungi Imperfecti, have been independently developed by a variety of species wholly unrelated and belonging to widely separated groups among the Pyrenomycetes, the Discomycetes and the Basidiomycetes. Such bulbiferous conditions, therefore, cannot in any sense be regarded as forming anything in the nature of a Natural Group. If one may judge from our actual knowledge of these forms, it would appear, on the contrary, that the bulbiferous condition was a specific one, the habit having been developed by certain species, only, in genera, the other members of which have no such secondary means of propagation: just as the habit of producing sclerotia of a characteristic type, has arisen in a few species, only, of Penecillium, like *P. Italicum*. The same principle is well illustrated in the large genus Corticium many species of which have been tested by means of pure cultures. Here again one finds a single species, only, which possesses the bulbiferous habit, namely *C. olutaceum*, pure cultures of which become completely covered by its dark brown bulbils.

In view of the wide distribution and common occurrence of bulbil-producing forms, it is not a little surprising to find such scanty references to them in mycological literature; and from the experiences of the writer in studying them, it seems certain that further attention to this subject will not only yield numerous other forms, but will show connections with "perfect" conditions even more varied than is at present indicated.

KEY TO THE SPECIES OF BULBILS HEREIN CONSIDERED.

According to their method of development bulbils may be grouped in three more or less well defined categories namely: those which originate from a primary spiral; those which develop from an intercalary primordium of several cells, and those which arise from a group of vertical hyphae. Using these characters as a fundamental basis for separation, the species above enumerated may be distinguished as follows.

Key to the Species of Bulbiferous Fungi.

A. Primordium normally involving more than one cell.
  I. Bulbils black or smoke-colored.
    1. Bulbils 75–100 μ in diam. margin even . . *Cabonia bulbifera.*
    2. " 200–300 μ " " " irregular *Papulospora pannosa.*
  II. Bulbils yellowish red to dark brown.
    1. Hyphae showing clamp-connections.
      1. Bulbils dark brown or chocolate colored.
        i. Bulbils 65–80 μ in diam. clamps conspicuous. *Corticium alutaceum.*
        ii. " 125–175 μ " " margin even, clamps inconspicuous . . . . *Papulospora anomala.*
      2 Bulbils yellowish or light brown.
        i. Bulbils light yellow, hyphae radiating conspicuously. *Grandinia crustosa.*
        ii Bulbils brownish yellow, hyphae formed evenly. "No 200."
    2. Hyphae not showing clamp-connections.
      1 Bulbils scanty, peritheeia usually present.
        i. Peritheeia with neck, lateral and terminal setae. *Melanospora cervicula.*
        ii. " " papilla and terminal setae. *Melanospora papillata.*
      2 Bulbils abundant, peritheeia usually absent.
        i. Primordium intercalary.
          (i) Bulbils brownish-yellow, central cells 28–55 μ in diam. . . . . *Papulospora immersa.*
          (ii). Bulbils straw-colored, central cells 10–20 μ in diam. . . . . *Papulospora irregularis.*
        ii. Primordium one or more lateral branches.
          (i). Primordium normally a single lateral branch.
            a. Primordium a spiral.
              § Cells heterogenous, definite cortex.
                A. One central cell.
                  * Cortex complete. *Papulospora parasitica.*
                  * * Cortex incomplete. *Acrospeira mirabilis.*
                B. More than one central cell.
                  * Spiral in one plane, cortical cells spinulose *Papulospora spinulosa.*
                  * * Spiral normally in more than one plane, 2–6 central cells.
                    α Bulbils dark brown. *Papulospora coprophila.*
                    β Bulbils brick red. *Papulospora rubida.*
              §§ Cells homogenous, bulbils 21–36 μ in diam. brownish producing sporotrichum spores. *Papulospora sporotrichoides.*

b. Primordium not a spiral.
§ Bulbils large, 100–750 μ, irregular. *Papulospora aspergilliformis.*
§§ " 70–150 μ, somewhat spherical, producing perithecia with slight papilla . . . *Melanospora anomala.*
(ii). Primordium two or more lateral branches forming a spherical aggregation of cells at the top. *Papulospora polyspora.*

III. Bulbils white to cream colored, 30–35 μ in diam. *Papulospora candida.*

IV. " steel gray, 21–36 μ in diam. . . . . *Papulospora cinerea.*

HARVARD UNIVERSITY
April, 1911.

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EXPLANATION OF PLATES.

The figures of Plates 1–12 were drawn with the aid of a camera lucida using different combinations of the Bausch and Lomb lenses. All the mature bulbils were drawn with the same magnification, namely 4 mm. objective and 3 eye piece, and for the stages of development of the bulbils, 4 mm. objective and 12 eye piece were used. The plates have been reduced in reproduction about three-quarters.

PLATE 1.

CUBONIA BULBIFERA.

FIGURES 1–6. Different forms of the primordium of the apothecium.
FIGURES 7, 8. Young apothecia.
FIGURE 9. Section of the mature apothecium.
FIGURE 10. Asci and paraphyses.
FIGURES 11–16. Stages in the development of the bulbil.
FIGURE 17. Mature bulbil.
FIGURE 18. Contortions of the hyphae.
FIGURE 19. Portion of a crushed bulbil with the contents of the cells escaping.
FIGURE 20. Ascospore.
FIGURE 21. The endosporium broken off.
FIGURES 22–24. Germinating Ascospores.
FIGURES 26, 27. Sprouting vegetative cells from the inner portion of the apothecium.
FIGURE 28. Germinating bulbil producing spiral primordia directly.

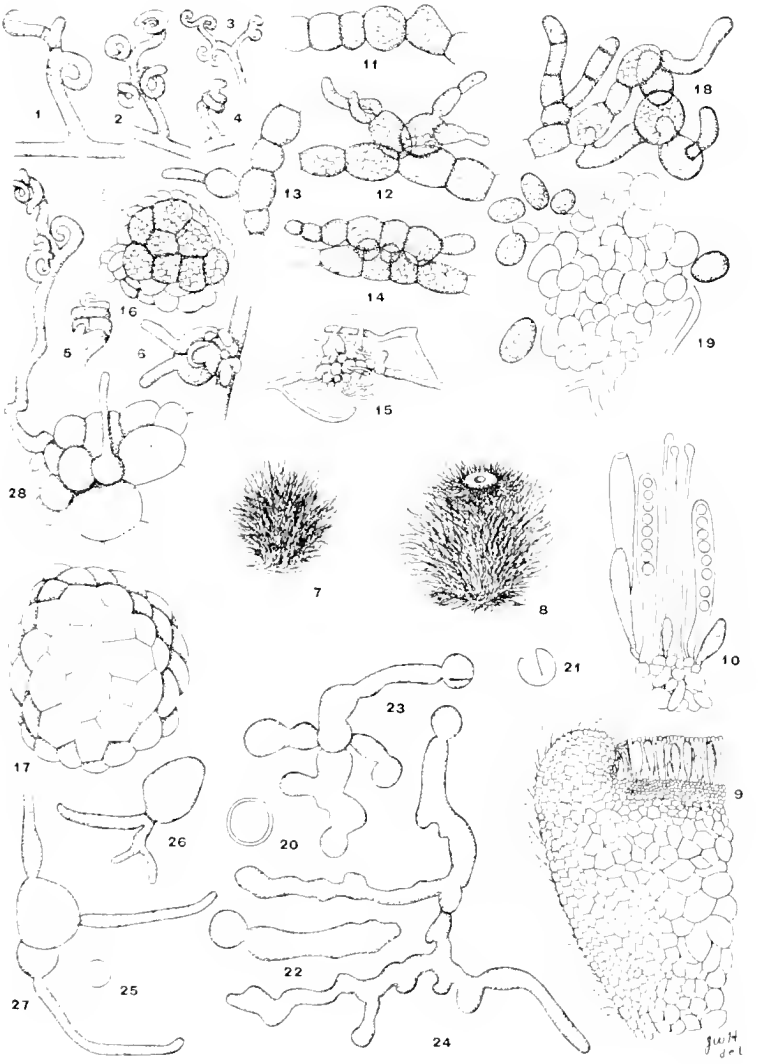
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PLATE 2.

MELANOSPORA PAPILLATA.

FIGURES 1–6. Stages in the development of the bulbil.
FIGURE 7. A group of Chlamydospore-like intercalary cells.
FIGURES 8–10. Stages in the development of the perithecium.
FIGURE 11. Outline of a mature perithecium showing the relative size of the bulbils.
FIGURE 12. A group of asci crushed from a young perithecium.
FIGURES 13–20. Germinating ascospores.
FIGURES 21, 22. Forms produced in Van Tiegham cell cultures.
FIGURE 23. Conidia on flask-shaped sterigmata produced on a hypha.
FIGURES 24, 25. Stages in the development of a terminal bulbil.
FIGURE 26. An intercalary bulbil with three large central cells.

MELANOSPORA ANOMALA.

FIGURES 27–30. Stages in the development of the bulbil.

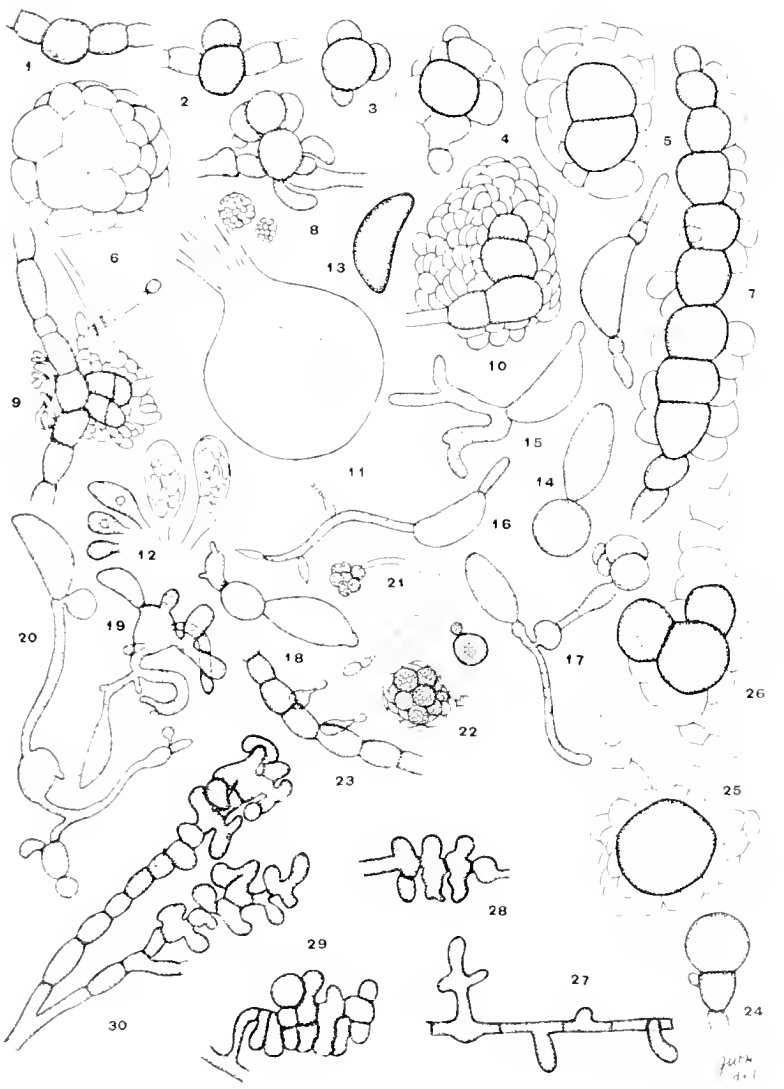
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PLATE 3.

Melanospora anomala.

Figures 1–12. Stages in the development of the perithecium.
Figure 12. Mature perithecium.
Figure 13. (a) Germinating ascospore showing a bottle-shaped sterigma.
(b) Bottle-shaped sterigma on a hypha.
Figures 14, 15. Other stages in the formation of the bulbil.
Figure 15. A mature bulbil.

Melanospora cervicula.

Figures 16, 17. Primordia of the bulbil.
Figure 18. A bulbil produced from a group of terminal cells.
Figure 19. Primordium of the perithecium and conidia on flask-shaped sterigmata.
Figure 20. Mature perithecium.
Figure 21. Abnormal forms common among the hyphae.
Figure 22. Chlamydospores of the Acremoniella type.
Figures 23, 24. "Harzia-like" fructification.

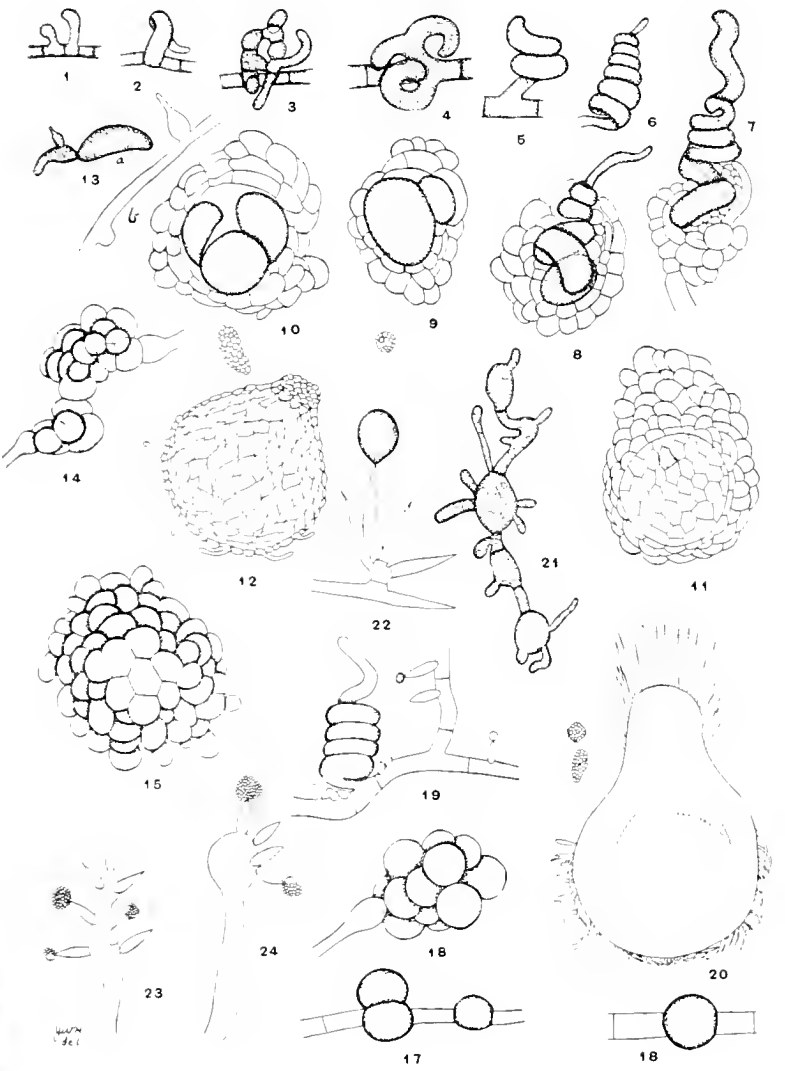
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PLATE 4.

PAPULOSPORA CANDIDA.

FIGURES 1, 2. Variation in the size of the conidia.
FIGURES 3–12, and 15–27. Stages in the germination of the conidia and the development of the bulbil from them.
FIGURES 28–41. Stages in the development of the bulbil from a lateral branch of the hyphae.
FIGURE 42. Germination of the superficial cells of the bulbil.
FIGURE 43. Conidiophores of Verticillium agaricinum var. clavisedum.
FIGURE 44. Portion of the hyphae showing large oil globules.
FIGURE 45. Showing intimate connection between the bulbil and the Verticillium.
FIGURE 46. An irregular primordium of a bulbil.
FIGURE 47. Ascoma of Geoglossum glabrum attacked by the parasite.

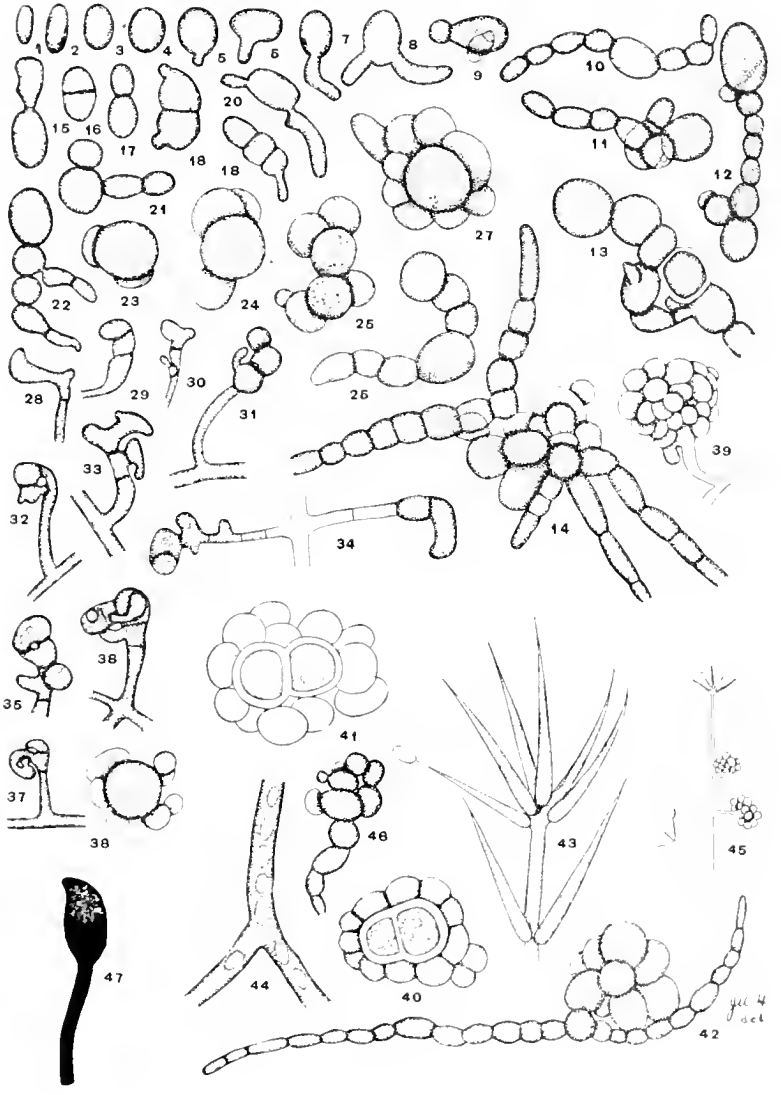
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PLATE 5.

Papulospora parasitica.

Figures 1–14. Show various stages in the development of the bulbil.
Figures 4, 5, & 9, 10. Show the protuberance from the lateral surface of the large central cell.
Figures 15, 16. Germinating bulbils.
Figure 17. Conidia-like bodies connected with the bulbil.
Figures 35b, 36. Swollen intercalary cells.

Acrospeira mirabilis.

Figures 18–23. Stages in the development of the bulbil.
Figure 20. The end-cell has enlarged to form the central cell.
Figure 21. The second cell has enlarged to form the central cell.
Figure 22. Several empty cortical cells are shown.

Reproductive Bodies Resembling Bulbils.

Figure 24–34. Irregular forms of a doubtful bulbil (No. 170).
Figure 35. Spore of Stephanoma strigosum Wallr.

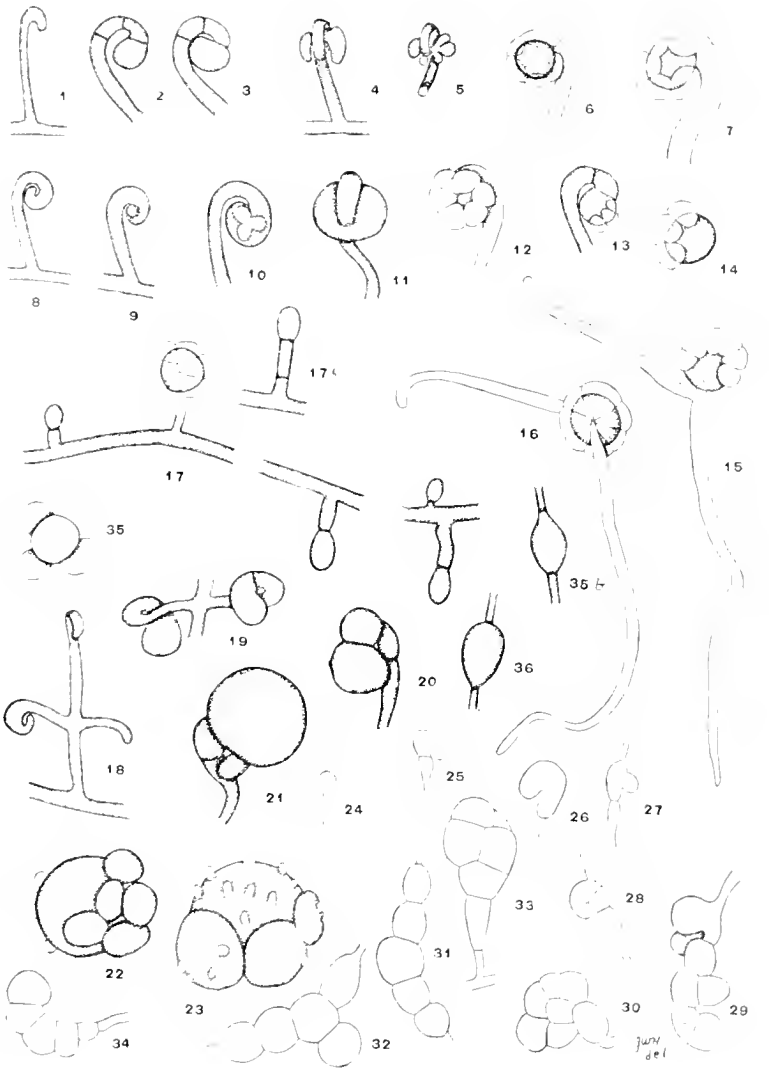
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PLATE 6.

Grandinia crustosa.

Figure 1. Pustulate habit of the fructification.
Figure 2. Hymenium with basidiospores.
Figure 3. Basidiospore.
Figures 4–10. Stages in the development of the bulbil.
Figure 10. Mature bulbil with the same magnification as all the other mature bulbils.

Papulospora anomala.

Figure 11–17. Stages in the development of the bulbil.
Figure 17. Mature bulbil.
Figure 18. Two primordia close together.
Figure 19. Large intercalary cells densely filled with oil globules.

Papulospora pannosa.

Figures 20–24. Stages in the development of the bulbil from intercalary cells.
Figure 25. Occasional mode of formation of intercalary primordia.

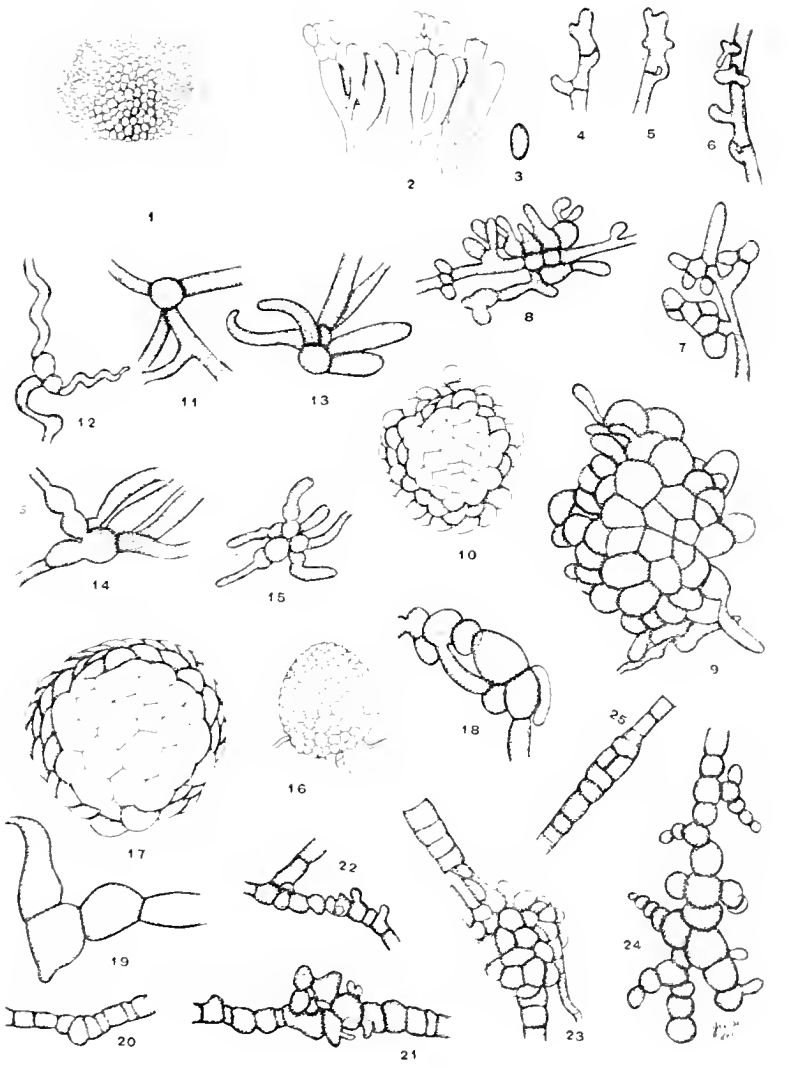
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PLATE 7.

PAPULOSPORA ASPERGILLIFORMIS.

FIGURES 1–4, & 6. Stages in the development of the bulbil.
FIGURE 5. A group of Chlamydospore-like bodies.
FIGURE 7. A primordium that produces a very irregular bulbil.
FIGURE 8. "Aspergillus-like" heads produced directly from the bulbil.
FIGURES 9–12. Different forms of the "Aspergillus-like" fructification.
FIGURE 12. Abnormal conditions.
FIGURES 13–16. Chlamydospores.
FIGURES 17, 18. Large swollen cells, likely storage cells.
FIGURE 19. Bulbil forming from terminal cells.
FIGURE 20. Section of a mature bulbil.

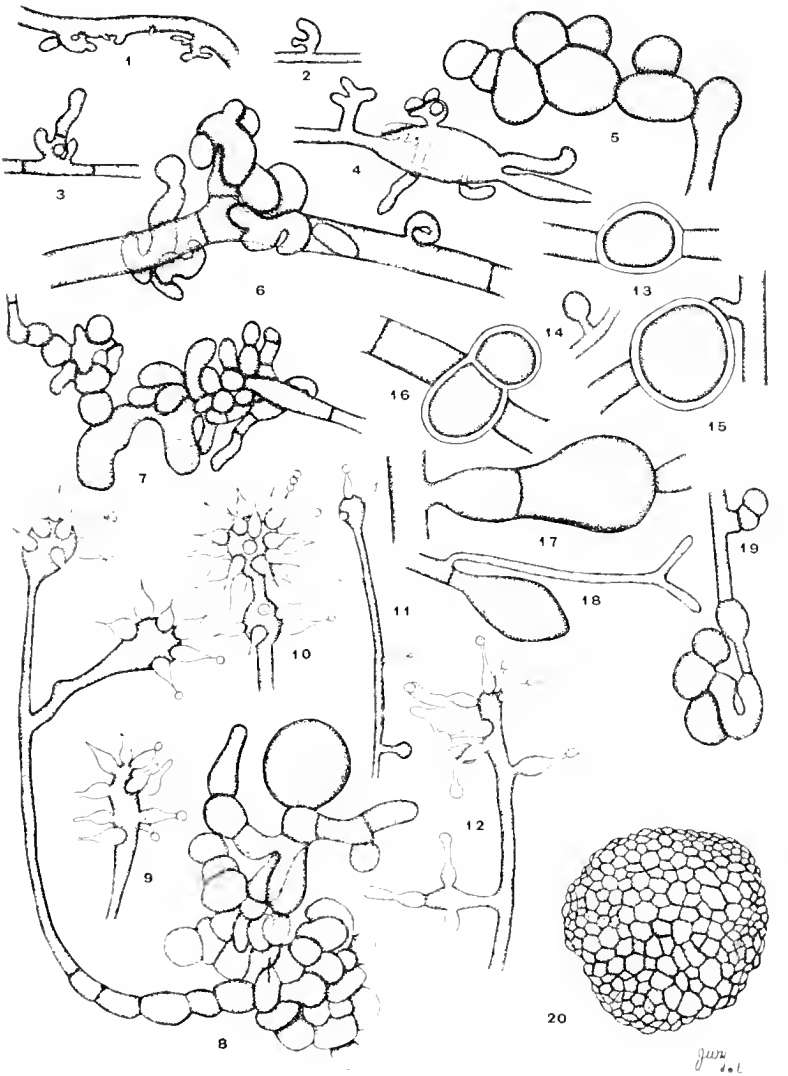
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PLATE 8.

Papulospora cinerea.

Figures 1–10. Stages in the development of the bulbil.
Figures 4, 6, & 9. Modifications of the regular mode of development.
Figures 10, 11. Mature bulbils.

Papulospora rubida.

Figures 12–16. Stages in the development of the bulbil.
Figures 25a–27a, 21, 22. Other stages in the development of the bulbil.
Figures 17, 20. The spiral primordium that sometimes occurs.
Figure 25. Section of a mature bulbil showing five large central cells.
Figure 18. Surface view of a mature bulbil.

Papulospora pannosa.

Figures 28–30. The development of a bulbil from a lateral branch.
Figure 31. A collapsed hypha showing rigid septa.

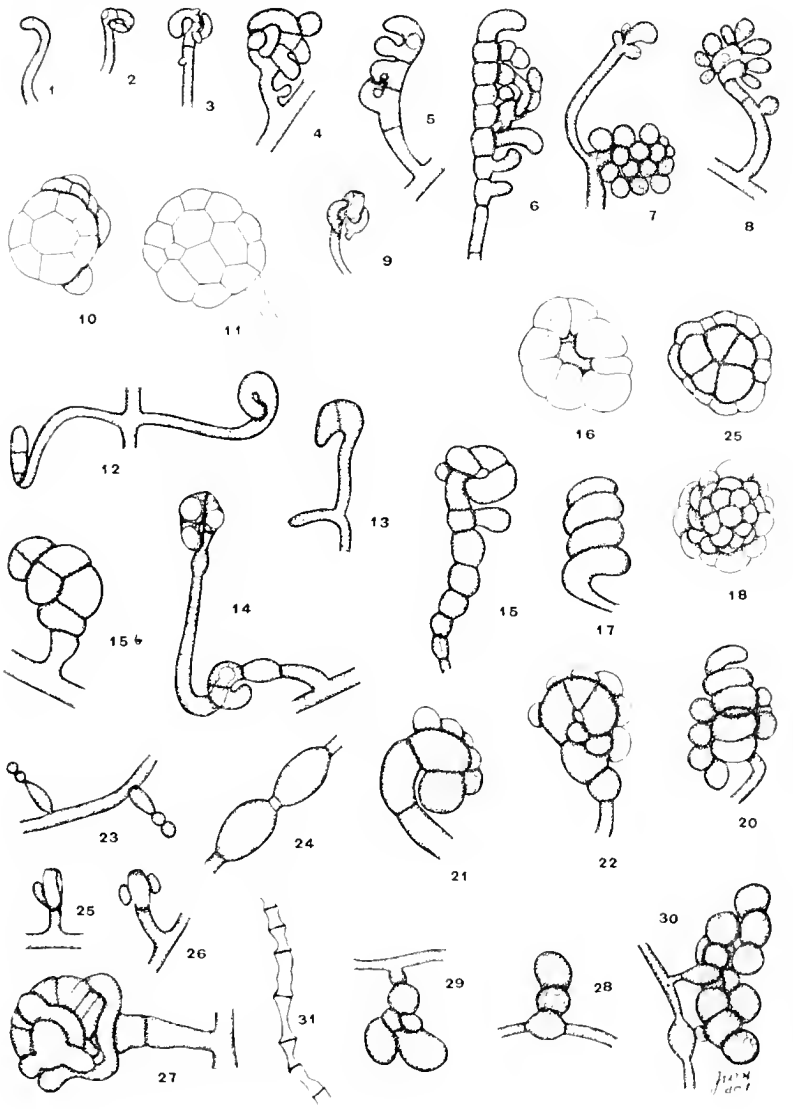
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PLATE 9.

PAPULOSPORA SPINULOSA.

FIGURES 1–7. Stages in the development of the bulbil.
FIGURE 8. Primordia produced from a superficial cell of an immature bulbil.
FIGURE 9. Section of a mature bulbil showing the "Annulus."
FIGURE 10. A surface view of the same looking down on the "Annulus."

PAPULOSPORA IRREGULARIS.

FIGURES 11–17. Stages in the development of the bulbil.
FIGURE 17. A mature bulbil.

PAPULOSPORA PANNOSA.

FIGURES 18–20. Stages in the development of the bulbil.
FIGURE 20. A mature bulbil.

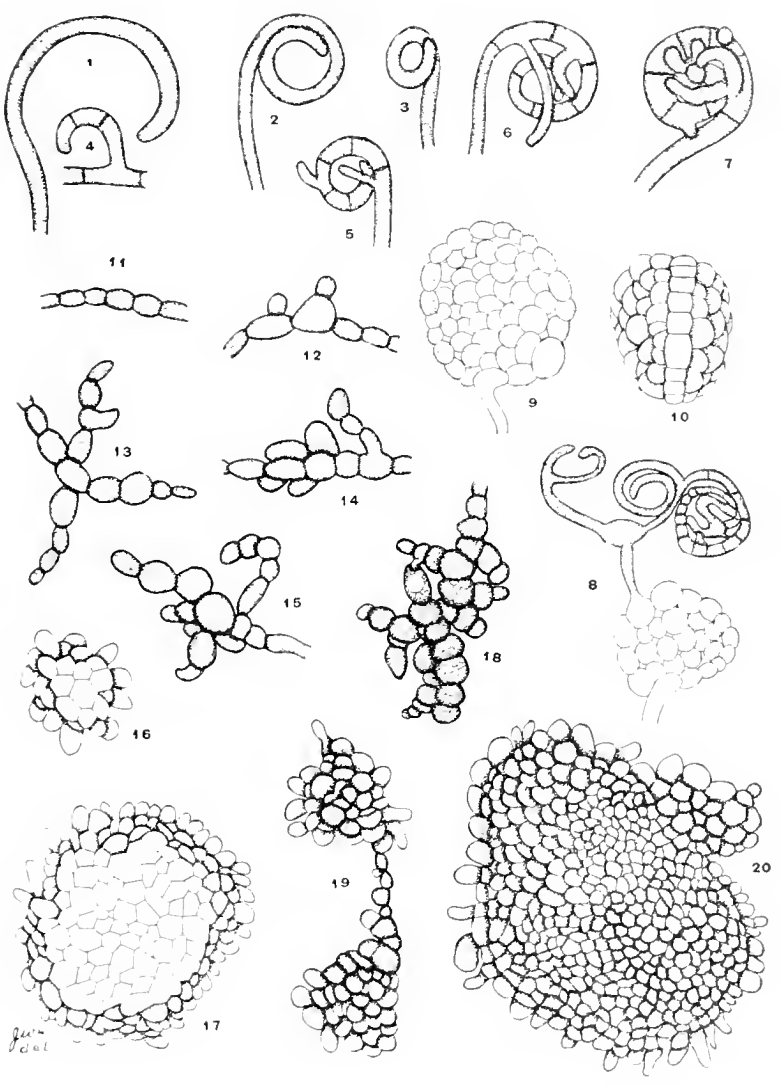
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PLATE 10.

Papulospora coprophila.

Figures 1–8. Stages in the development of a bulbil from a spiral.
Figure 6. An unusual condition, the production of conidia directly from the spiral.
Figure 8. A spiral primordium surrounded by an irregular layer of cells.
Figure 9. Immature bulbil that has developed like Figs. 14 and 15, and also a spiral primordium.
Figure 10. Median section of a mature bulbil with two large central cells.
Figure 11. A mature bulbil with the contents of the large cells crushed out (Fig. 11b).
Figure 12. Germination of one of these cells.
Figures 13–15. Forms arrested in the process of development.
Figures 16. Surface view of the mature bulbil.

Papulospora immersa.

Figure 17. Irregular hypha densely filled with protoplasm. The primordium of the bulbil.
Figure 18. Primordium consisting of a single intercalary cell.
Figure 19–25. Stages in the development of the bulbil.

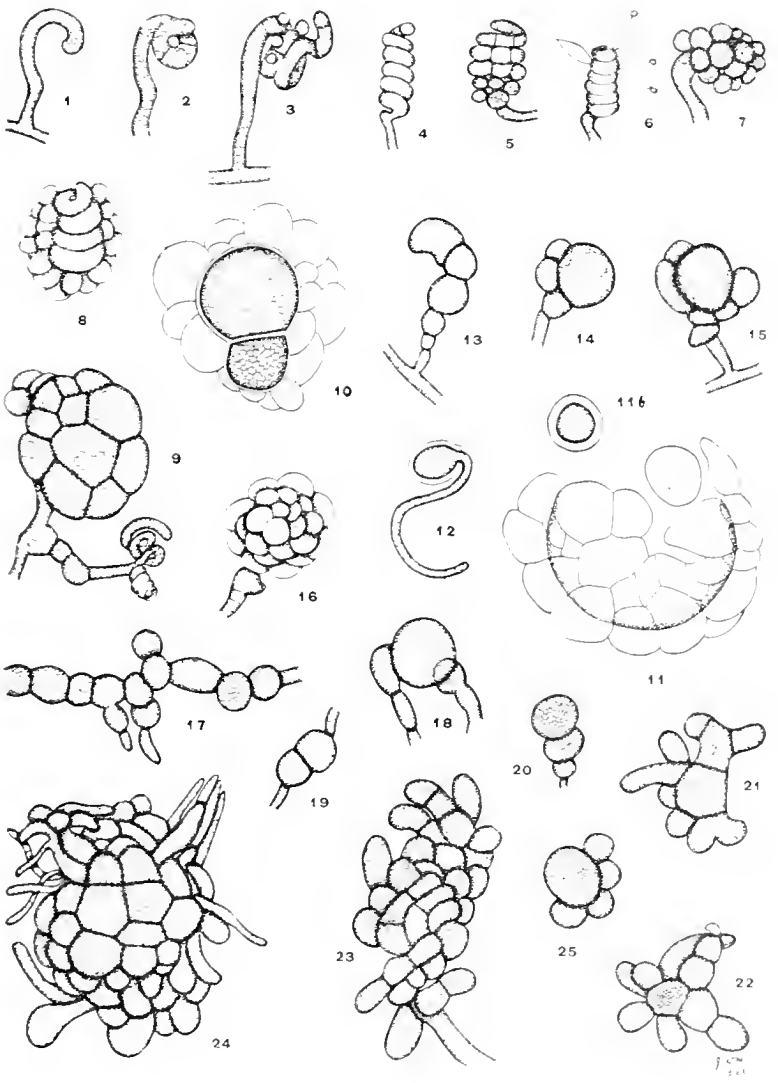
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PLATE **11**.

PAPULOSPORA POLYSPORA.

FIGURES 1–7. Stages in the development of the bulbil.
FIGURE 7. A mature bulbil.
FIGURE 8. Group of spores adhering loosely together.
FIGURES 9 & 10. Germinating spores.
FIGURES 9b, 10b, 11–13. Modifications that occur when grown in liquid media.

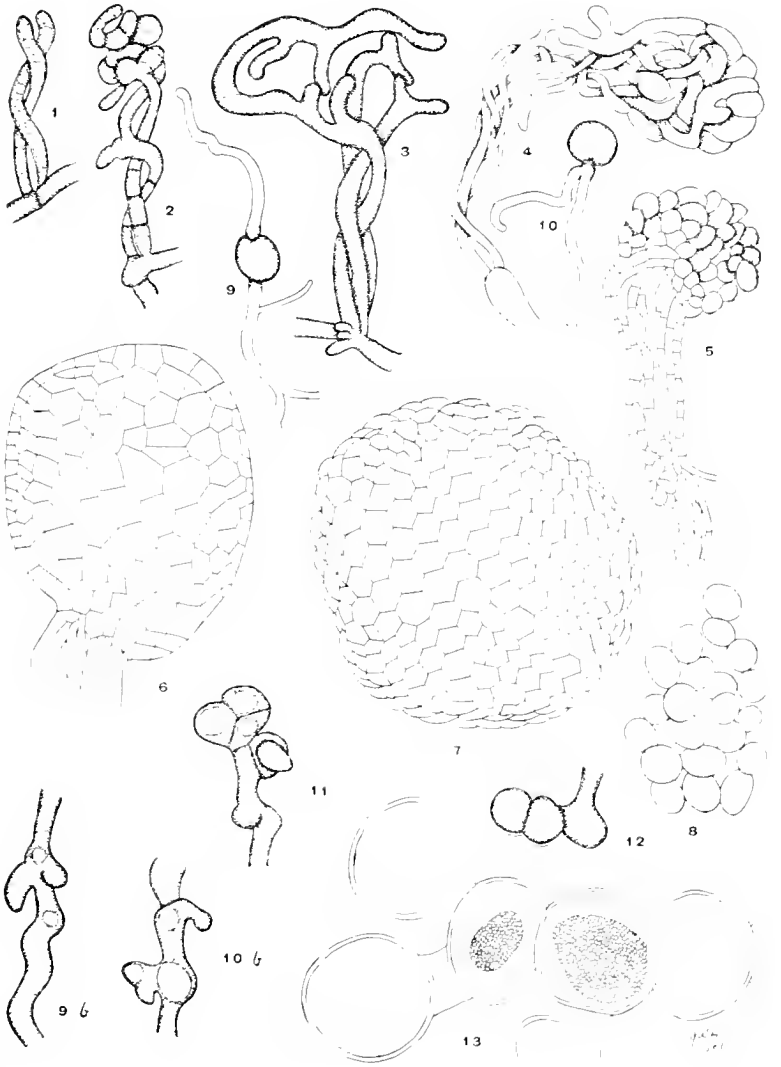
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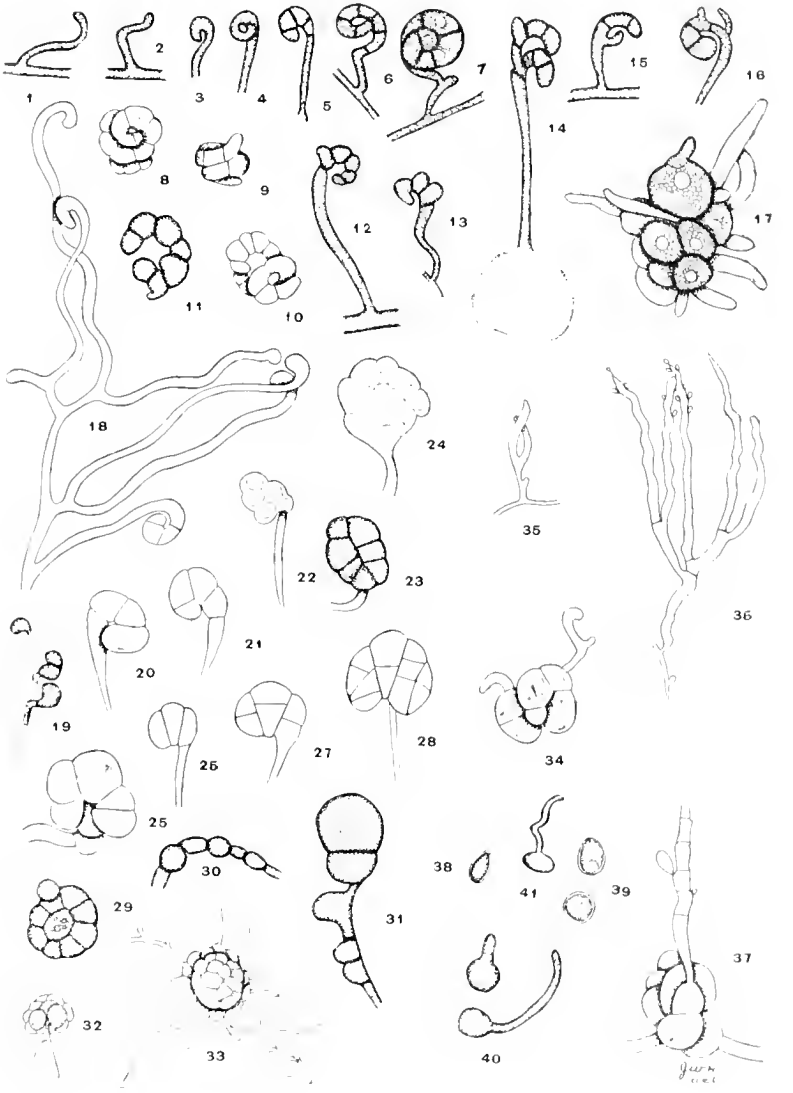
PLATE 12

PAPULOSPORA SPOROTRICHOIDES.

FIGURES 1–9. Stages in the development of the bulbil.
FIGURE 8. A mature bulbil
FIGURE 9. A side view of an immature bulbil.
FIGURES 10, 11. Abortive forms.
FIGURES 12–16. Modifications in the formation of the spiral.
FIGURE 17. An irregular bulbil germinating, magnified more than the others.
FIGURE 18. Branch of the hyphae showing primordia of the bulbils.
FIGURES 19–25. Modifications in the development of the bulbils which are hyaline.
FIGURES 26–28. Semi-diagrammatic representation of the mode of cell formation in the development of the hyaline bulbils.
FIGURE 29. A section of a mature bulbil.
FIGURES 30, 31. Large intercalary and terminal cells found in the hyphae.
FIGURES 32–34. Germinating bulbils.
FIGURES 25–26. Conidiophores with conidia.
FIGURE 37. Conidiophore produced directly from the bulbil in a Van Tieghem cell culture.
FIGURE 38. Conidium.
FIGURE 39. The form the conidia usually assume before germinating.
FIGURES 40, 41. Germinating conidia.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